# PROTEIN UTILIZATION BY POULTRY

*British Egg Marketing Board Symposium Number Two*

# PROTEIN UTILIZATION BY POULTRY

*Edited by*

R. A. MORTON

AND

E. C. AMOROSO

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# FOREWORD

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> To promote the scientific study of domestic fowl by drawing attention to its importance, by stimulating the recruitment and training of research workers, by organising basic and applied research in poultry husbandry and in the physiology, pathology, genetics, nutrition and biochemistry of fowl, and by facilitating communication between those working in this field.

In the course of its first five years the Committee has made numerous recommendations to the Board, all of which have been accepted. In facilitating communication, in accordance with its final instruction, the Committee obtained support for the journal, *British Poultry Science*, and published a directory of scientists and institutions concerned with poultry.

The aims of the Symposia of which this is the second, are to enable those working in the field to meet each other, to promote the exchange of information and provide an opportunity for reviewing the current state of knowledge in particular aspects of poultry science. It is hoped further that the Symposia will indicate where further effort could most fruitfully be concentrated.

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# PREFACE

This book is the record of a second Symposium organised by the Scientific Advisory Committee of the British Egg Marketing Board and held at the University of Nottingham, School of Agriculture, Sutton Bonington (22 and 23 September, 1965). The previous Symposium, also held at Sutton Bonington (16 to 18 December, 1964) dealt with the physiology of the domestic fowl and has been published as *British Egg Marketing Board Symposium, Number 1* (Oliver and Boyd, Edinburgh and London, 1966). The present work is being issued in the same format and it is hoped to arrange for accounts of further symposia to appear annually.

This new symposium on protein utilization by poultry was largely planned by Dr H. Temperton and the arrangements for the meeting were made by Dr N. R. Knowles, Secretary of the Scientific Advisory Committee. As on the previous occasion the success of the conference owed much to the presence of overseas visitors. Papers by Dr C. Calet of Jouy-en-Josas, France, Professor J. D. Summers of Guelph, Canada, Professor G. F. Combs of Maryland, U.S.A., Professor D. C. Snetsinger of Minnesota, U.S.A. and Professor J. McGinnis of Washington State University, U.S.A., were all much appreciated and gave rise to lively discussion. Dr Calet's paper, admirably presented in English, was prepared for publication in French and kindly translated into English by Dr T. C. Carter. Two-thirds of the papers read were nevertheless by British workers whose contributions, together with the general discussions, showed that interest in the subject in this country was lively, widespread and informed. It was clear that the participants generally, while fully cognizant of the hard core of fundamental nutritional science, looked at the bird, and particularly the laying hen, as giving rise to special problems. The provision of dietary protein was seen as part of the wider problem of matching diet to need over the whole life-cycle, and methods of ensuring appropriate intakes of essential amino acids were discussed very realistically.

Much has been done in the sixty years since E. G. Willcock and F. G. Hopkins, experimenting on mice, added tryptophan to a dietary in which zein was the sole protein (*J. Physiol.*, 1906-07, 35, 88). They pointed out that zein, unsupplemented, was unable to maintain growth in young mice and that although addition of tryptophan prolonged the survival period and increased well-being it did not make the diet adequate for growth. T. B. Osborne and L. B. Mendel (Carnegie Institution, Washington, 1911, Publication 156, Parts I and II) carried out on rats their classical feeding experiments with isolated food

substances, e.g. single purified proteins such as casein. It is interesting to recall how clearly they demonstrated suspension of growth on a maintenance diet, and how excellent was their strategy.

> We have stated that by our plan a biological comparison of different proteins in respect to their role in growth can at length be made. Our work in this direction must be regarded as barely begun. Nevertheless it is of interest to speculate as to the indications already gained and the outlook for future work. A comparison of the two groups of proteins—those adequate and those inadequate for growth purposes—at once reveals the fact that the latter category comprises proteins (gliadin, hordein, zein) commonly spoken of as chemically "incomplete". They lack one or more of the amino-acid complexes which are obtainable from the so-called "complete" proteins. None of them furnish glycocoll or lysine, and zein in addition is devoid of tryptophan. By feeding relatively small quantities of proteins like casein with gliadin growth begins at once. Here we can determine the minimum of suitable protein to satisfy this growth requirement. . . . The addition of amino acids to "complete", as it were, the inadequate proteins can now be studied amid controllable factors; the biological role of hydrolyzed proteins and the significance of complete hydrolysis or digestion in nutrition can be examined anew.

This work was followed in due course by that of W. C. Rose and his colleagues. In their feeding experiments with mixtures of highly purified amino acids, a high point was reached (McCoy, Meyer and Rose, *J. Biol. Chem.*, 1935-36, 112, 283) with the isolation and identification of threonine. This perhaps marked the end of an epoch.

The volume of research work published more recently is so large that the most fruitful advances are not yet so readily discernible. Biochemical discoveries, indicating complicated interrelationships between the metabolism of carbohydrates, fats, vitamins, proteins and minerals make necessary 'careful attention to the composition of experimental diets'! As A. A. Albanese and L. A. Orto (*Newer Methods of Nutritional Biochemistry*, Academic Press, 1963) also said, 'excessive artificiality may lead to results of limited practical usefulness'. Thus, these authors remind us that failure to provide a reasonable intake of non-essential amino acids has resulted in an apparent need for a caloric intake 50% above 'normal'.

It is very noticeable that the various schools of poultry research represented in the present volume all have their feet firmly on the ground. The great interest in available lysine value is evidence of such realism. Although the continuing need for interplay between 'pure' and 'applied' research was treated as axiomatic there was no complacent assumption that technological advance results automatically

from pure research. One sensed instead a realisation that fully efficient use of dietary protein by the laying hen is a harsh economic necessity in the modern world threatened as it is by a human population avalanche.

A minor editorial problem has emerged very clearly from preparing the present volume for publication. In future symposia speakers contributing impromptu remarks to general discussions should be invited and indeed pressed to leave a written version of their comments with the organizers before the end of the conference. On this occasion the tape-recordings were not always intelligible to the typists (partly because of 'noises off') and sometimes speakers could not be identified. Tribute must be paid to the secretaries who transcribed the recordings and retyped the much-edited versions.

R. A. M.

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# 1

# METHODS OF EVALUATING THE AMINO ACID CONTENT OF FEEDINGSTUFFS AND THEIR LIMITATIONS

E. L. MILLER

*School of Agriculture, University of Cambridge*

*Synopsis*

Ion exchange methods and microbiological methods for measuring the total amino acid content of feedingstuffs are reviewed with particular reference to the destruction of nutritionally important amino acids during acid hydrolysis. Evidence is presented that the lysine, methionine and other amino acids may be, in part, unavailable in commercial meat and fish meals, and also that heat treatment of proteins reduces availability of these amino acids. Chemical and microbiological methods for the determination of the 'available' content of amino acids are reviewed. Results obtained by these laboratory procedures are compared with those based on biological assays using rats or chicks.

## *Introduction*

THE purpose of this symposium is to discuss current knowledge, of the amino acid requirements of different classes of poultry, of the way in which these requirements vary with other nutrient components of the diet and of methods used to evaluate feedingstuffs so that rations can be compounded containing the required amino acid levels. Thus, on the one hand we have requirement standards for the individual amino acids and on the other hand total amino acid analysis of feedstuffs which has now become almost routine in many feed compounder and research laboratories. The problems considered here are to ascertain:

(1) the accuracy of total amino acid analyses;

(2) whether the nutritive value of the protein predicted from the total amino acid content agrees with the biologically determined value; and

(3) the usefulness of alternative methods of analysis for 'available' amino acid content.

## *Accuracy of Total Amino Acid Analysis*

Discussion here is restricted to microbiological methods and to ion exchange chromatographic methods, although other chromatographic and chemical methods are available for specific amino acids.

The automated chromatographic procedures offer the advantages of greater reproducibility and the simultaneous analysis of nearly all the amino acids in a protein hydrolysate. Certainly, amino acid mixtures can be estimated with an accuracy of 100±3% (Moore, Spackman & Stein, 1958) compared with 100±10% usually accepted for microbiological assays. However, the analysis of feedstuffs requires the additional procedure of acid hydrolysis and much greater variability and even bias can be introduced at this stage. For chromatographic analysis the protein must be completely hydrolysed, but the conditions necessary to bring about cleavage of the most resistant peptide bonds also cause destruction of some amino acids. No single time of hydrolysis is satisfactory for all amino acids (Peters, 1960; Mahowald, Noltmann & Kuby, 1962) although Krampitz (1960) has reported that by using stannous chloride as a catalyst and 3N instead of 6N hydrochloric acid, hydrolysis of even the resistant peptides of valine, leucine and isoleucine is complete in 24 hours and destruction of serine and threonine is decreased. However, even under these milder conditions, cystine is partially and tryptophan almost completely destroyed. Methionine may also be partially destroyed during hydrolysis, particularly with materials rich in carbohydrate. To correct for this destruction, Schram, Dustin, Moore and Bigwood (1953) calculated methionine from the sum of recovered methionine, methionine sulphoxide and a positive correction factor of 20% of the methionine sulphoxide content.

In practical feed compounding it is found that if diets are formulated to meet the sulphur amino acid and lysine requirements then the needs for other amino acids will usually be met, although tryptophan and threonine may be borderline in some instances. Thus the amino acids which present the greatest analytical difficulties—cystine, methionine, tryptophan and threonine—are also those which nutritionally are the most important. Indeed many analyses have been reported giving results for all the amino acids with the exception of cystine and tryptophan. Such data are of limited use in the practical situation of feed formulation. However, chromatographic methods are available for the specific determination of some of these nutritionally important amino acids. Lysine may be separated on a 15-cm cation exchange column using pH 5·28 buffer; cystine and methionine residues may be oxidized to the stable cysteic acid and methionine sulphone prior to hydrolysis and then eluted from a 100-cm cation exchange column with pH 2·4 buffer ahead of the other amino acids (Bujard & Mauron, 1963). The determination of cystine as cysteic acid is probably the most reliable method available, but recoveries of methionine as methionine sulphone have been reported to range from 84% (Bunyan & Woodham, 1964) to 100% (Bidmead & Ley, 1958; Moore, 1963).

Microbiological methods are particularly suited to the routine determination of a few selected amino acids in many samples. Hydro-

lytic conditions can be selected to suit the amino acid being assayed. Hydrolysis times of 8 hours for methionine and 12 hours or longer for other amino acids, and alkaline hydrolysis for tryptophan are generally employed. Mild conditions of acid hydrolysis followed by assay with the proteolytic *Streptococcus zymogenes* were used by Ford (1962) to measure 'total' levels of amino acids. With carefully selected conditions of partial hydrolysis the results obtained are maximum values, since increasing the severity of hydrolysis results in progressively lower values (Waterworth, 1964). The lower results obtained after more

TABLE 1

*Mean values with standard deviation of amino acid content (g/16 g nitrogen) of three preparations of cod muscle carried out in six different laboratories*

| Heat treatment | Cod 23 unheated control | Cod 35 27 h, 116°, 14% water | Cod 25 27 h, 85°, 14% water (+10% glucose) |
|---|---|---|---|
| Cystine | 1·1 ±0·07 (5)* | 0·45±0·05 (4) | 0·8 ±0·07 (3) |
| Methionine | 3·5 ±0·12 (5) | 3·4 ±0·12 (3) | 3·1 ±0·00 (2) |
| Aspartic acid | 9·7 ±0·77 (5) | 10·0 ±0·94 (4) | 10·2 ±0·84 (4) |
| Threonine | 4·4 ±0·71 (5) | 4·5 ±0·83 (4) | 4·8 ±0·99 (4) |
| Serine | 4·1 ±0·77 (5) | 3·7 ±0·46 (4) | 4·3 ±0·32 (4) |
| Glutamic acid | 14·6 ±2·44 (5) | 15·3 ±1·92 (4) | 14·8 ±2·73 (4) |
| Proline | 3·5 ±0·12 (3) | 4·0 ±0·36 (2) | 4·1 ±0·99 (2) |
| Glycine | 4·3 ±0·53 (5) | 4·3 ±0·24 (4) | 4·3 ±0·30 (4) |
| Alanine | 5·9 ±0·69 (5) | 6·1 ±0·36 (4) | 5·6 ±0·72 (4) |
| Valine | 4·9 ±0·65 (5) | 5·0 ±0·48 (4) | 5·0 ±0·44 (4) |
| Isoleucine | 4·5 ±0·51 (5) | 4·8 ±0·37 (4) | 4·6 ±0·39 (4) |
| Leucine | 8·0 ±0·73 (5) | 8·1 ±0·22 (4) | 8·2 ±0·31 (4) |
| Tyrosine | 3·5 ±0·19 (4) | 3·6 ±0·10 (2) | 3·8 ±0·27 (3) |
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| Lysine | 9·3 ±0·60 (6) | 8·6 ±0·18 (5) | 6·0 ±0·56 (5) |
| Histidine | 2·2 ±0·25 (5) | 2·5 ±0·41 (4) | 2·1 ±0·17 (4) |
| Arginine | 6·1 ±0·34 (5) | 6·2 ±0·31 (4) | 4·8 ±0·33 (4) |
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complete hydrolysis may be attributed to destruction of either amino acids or of growth stimulating peptides. For some materials the method has given unexpectedly high values—e.g. 4·0 g methionine/16 g nitrogen in skim milk powders and a white-fish meal (Ford, 1962)—and this deserves further study.

In a recent study of the total amino acid composition of control unheated cod muscle and of two heated preparations, chromatographic analyses were carried out in six different laboratories, although not all the laboratories analysed every material for all the amino acids. The techniques employed included analysis of hydrolysates of unoxidized and oxidized protein and the use of manual as well as automatic equipment. The results are summarized in Table 1. Detailed results

The automated chromatographic procedures offer the advantages of greater reproducibility and the simultaneous analysis of nearly all the amino acids in a protein hydrolysate. Certainly, amino acid mixtures can be estimated with an accuracy of 100±3% (Moore, Spackman & Stein, 1958) compared with 100±10% usually accepted for microbiological assays. However, the analysis of feedstuffs requires the additional procedure of acid hydrolysis and much greater variability and even bias can be introduced at this stage. For chromatographic analysis the protein must be completely hydrolysed, but the conditions necessary to bring about cleavage of the most resistant peptide bonds also cause destruction of some amino acids. No single time of hydrolysis is satisfactory for all amino acids (Peters, 1960; Mahowald, Noltmann & Kuby, 1962) although Krampitz (1960) has reported that by using stannous chloride as a catalyst and 3N instead of 6N hydrochloric acid, hydrolysis of even the resistant peptides of valine, leucine and isoleucine is complete in 24 hours and destruction of serine and threonine is decreased. However, even under these milder conditions, cystine is partially and tryptophan almost completely destroyed. Methionine may also be partially destroyed during hydrolysis, particularly with materials rich in carbohydrate. To correct for this destruction, Schram, Dustin, Moore and Bigwood (1953) calculated methionine from the sum of recovered methionine, methionine sulphoxide and a positive correction factor of 20% of the methionine sulphoxide content.

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from three laboratories have been published (Miller, Hartley & Thomas 1965), showing that the average 'between-laboratory' coefficient of variation was 9%. This is somewhat greater than the variability found within a laboratory; for example, Ellinger and Boyne (1965) obtained a coefficient of variation of approximately 3% in the quadruplicate analysis of cod fillets. A collaborative trial of ion-exchange chromatography of pure amino acid mixtures, conducted before the days of automated equipment (Bender, Palgrave & Doell, 1959), indicated a considerable inter-laboratory variability and enabled individual laboratories to improve their technique. A similar collaboration to determine the amino acid content of a range of feedingstuffs, using the newer techniques, may be equally instructive.

### *The Concept of Amino Acid Availability*

The total amino acid content is a measure of the potential nutritional value. With certain feedstuffs, however, the full value of an amino acid may not be obtained even though that amino acid is the limiting factor in the diet. Barnes and Kwong (1964) have discussed a large number of factors, such as incomplete digestibility and bacterial deamination in the intestinal tract, which could operate to reduce the value of dietary amino acids. In order to cover all such possibilities we, at Cambridge, have preferred to define the available content of an amino acid as the potency of the feed under test, relative to the free amino acid, to support growth of an animal under conditions when the amino acid is the only limiting factor in the diet. Under these conditions the values obtained are net values dependent upon the interaction of the test protein, the animal and the experimental conditions used; in certain instances the values obtained are less than the digestible amino acid content, measured by analysis of food and faeces, indicating that factors other than incomplete digestibility may be involved.

That there is a problem of incomplete amino acid availability is illustrated by recent work on the effect of heat on cod muscle. Heating cod muscle for 27 hours at 116° resulted in a loss of approximately 60% of total cystine and 6% of total lysine, but no significant loss of any other amino acid. Heating cod muscle mixed with 10 parts by weight of glucose for 27 hours at 85° resulted in losses of total cystine, lysine and also of arginine (Table 1). Chemical scores calculated from these analyses by comparison with the amino acid requirements of the rat (Bender, 1958) are shown in Table 2 along with the determined Net Protein Ratio of the materials for the rat (Miller, Carpenter & Milner, 1965). Clearly the changes in nutritive value have not been paralleled by changes in total amino acid composition. On the other hand, available methionine determined by bioassays with the chick decreased markedly on heat treatment, and closely paralleled the results of the rat test (Table 2). Other studies of the effect of heat on

protein and protein carbohydrate mixtures (reviewed by Miller, Hartley & Thomas, 1965) have all shown similar considerable losses of nutritive value accompanied by only small changes in total content of amino acids. In a recent collaborative trial two Peruvian fish meals similar in total amino acid composition, but very different in protein quality for both rats and chicks (Bunyan & Woodham, 1964) were also found to be significantly different in value as protein supplements in practical-type pig diets (Barber, Braude, Chamberlain, Hosking & Mitchell, 1964).

Attention has been focused on the availability of lysine ever since the demonstration that the damaging effects of mild heating of protein-carbohydrate mixtures could be rectified by lysine supplementation

TABLE 2

*Calculated chemical score, net protein ratio and available methionine (g/16 g nitrogen) of unheated and heated cod muscle*

| Material | Chemical Score | Net protein ratio | Methionine available to the chick |
|---|---|---|---|
| C 23 unheated control | 92* | 4·6 | 3·4 |
| C 35 27 h, 116°, 14% water | 79* | 2·9 | 2·2 |
| C 25 27 h, 85°, 14% water (+10% glucose) | 79* | 0·69 | 0·4 |

* Cystine+methionine calculated to be limiting.

(Henry, Kon, Lea & White, 1947-8). Lea and Hannan (1950) showed the ε-amino group of lysine reacted with carbonyl groups of reducing sugars and it is presumed that this bound lysine is unavailable. Measurement of lysine residues with free ε-amino groups by reaction with fluorodinitrobenzene (FDNB) has been used as an indicator of processing damage of carbohydrate-rich materials such as biscuits and dried milks (Carpenter & March, 1961; Wiechers & Laubscher, 1961).

In other studies, heating of proteins containing little carbohydrate also resulted in reduced reactivity of lysine to FDNB, but the degree of heat needed to bring about damage was much greater than when reducing sugars were present (Carpenter, Ellinger, Munro & Rolfe, 1957; Lea, Parr & Carpenter, 1960; Carpenter, Morgan, Lea & Parr, 1962). Furthermore, the FDNB-available lysine of some 60 commercial samples of meat, fish and whale meat meals was found to correlate with the Gross Protein Value for chicks, an assay which for most materials is a reflection of the available lysine content of the protein (Boyne, Carpenter & Woodham, 1961).

Much less attention has been paid to the availability of other amino acids. This is probably because there are no obvious possibilities of chemical binding of amino acids such as methionine or the even more inert valine, leucine and isoleucine. However, the range in protein

quality found for meat, fish and whale meat meals when fed as the sole protein source either to rats (Boyne *et al.*, 1961) or to chicks (Kratzer & Davis, 1959), conditions under which the sulphur amino acids are usually limiting, is much greater than the range in total cystine and methionine. Thus the sulphur amino acids must be partially unavailable in the poorer quality meals. This has been confirmed by concomitant sulphur and nitrogen balance studies with rats which showed the digestibilities of S and N to range from 53% to 90% in meals selected to represent a wide range in protein quality (Miller & Carpenter, 1964). Bioassays of the same samples with the chick for methionine gave values ranging from 0·8 to 3·1 g/16 g nitrogen representing availabilities of from approximately 50% to 100% (Miller, Carpenter, Morgan & Boyne, 1965).

Supplementing good-quality meat and fish meals with sulphur amino acids often results in a great increase in quality, even up to that of whole egg (Hoagland, Ellis, Hankins & Snyder, 1948; Hoagland, Ellis, Hankins, Snyder & Hiner, 1951). On the other hand, the value of poor-quality meals is often only slightly increased by sulphur amino acid supplementation; after supplementation the value does not approach that of supplemented good-quality meals and even additions of several other amino acids have little effect (Miller, 1956; Miller & Carpenter, 1964; Smith & Scott, 1965). The implication here is that the availability of all the amino acids is greatly reduced in the poor-quality meals. So far, only few bioassays for amino acids other than lysine and methionine have been carried out with such materials. These studies are needed to indicate the extent of unavailability that may be encountered in commercial meals. For example, a chick bioassay of heated cod muscle for isoleucine has indicated a 20% reduction in availability compared with a 30% decrease in available methionine (Nesheim & Carpenter, unpublished results).

### *Methods of measuring Available Amino Acids*

(a) *Bioassays.* The definition given of available amino acid content limits the direct measurement of available amino acids to bioassays in which weight gain, feed conversion efficiency or nitrogen retention is the response used to measure growth. However, bioassays cannot be regarded as suitable for routine evaluation, but are necessary to provide results against which chemical, enzymic or microbiological procedures suitable for routine application, may be assessed. The problems of bioassays have been reviewed by Harper and de Muelenaere (1963), and further studies and improvement in techniques are required so that greater confidence may be placed in these primary standard results.

(b) *Chemical procedures.* The FDNB-available lysine method of Carpenter (1960) has now been widely tested and found to be a useful

predictor of protein quality of animal protein concentrates. Pritchard, McLarnon and McGillivray (1964), reporting the results of their routine analysis of over 750 samples of meat and bone meals and 250 samples of fish meals, gave values (g/16 g nitrogen) ranging from 3·2–5·2 for meat meals, 4·1–8·3 for white fish meals, 5·5–8·1 for herring meals and 4·7–7·3 for Peruvian fish meals. Also Olley and Watson (1961) have reported a range of 3·9–8·2 in 21 samples of Peruvian fish meals.

With vegetable protein concentrates hydrolysis causes losses of between 20 and 30% of added dinitrophenyl (DNP)-lysine (Carpenter & March, 1961; Butterworth & Fox, 1963) and with cereals losses are even greater (Carpenter & Milner, unpublished data). In addition some soluble humins, formed during hydrolysis of carbohydrate-rich materials, are not separated from DNP-lysine by the ether extraction, or by the reaction with methyl chloroformate used in the Carpenter (1960) procedure, and may contribute to the available lysine value (Milner, 1963). Alternative methods involving chromatographic separation of DNP-lysine have been proposed (Baliga, Bayliss & Lyman, 1959; Rao, Carter & Frampton, 1963) and have been used to study effects of processing of soya beans (Ascarelli & Gestetner, 1962; Ben-Gera & Zimmerman, 1964). None of these procedures can be used as the sole test to predict the protein quality of commercial oil seeds where varying levels of toxins such as trypsin inhibitors and gossypol may occur (Ascarelli & Gestetner, 1962).

Neither of the alternative procedures overcomes the problem of loss of DNP-lysine during hydrolysis of carbohydrate-rich materials. This has led Mauron and Bujard (1963) to measure lysine with free ε-amino groups by reaction with o-methylisourea. Lysine is converted to homoarginine which is liberated without loss on acid hydrolysis and is separated and estimated by ion-exchange chromatography. The method has the advantage of giving total amino acids and available lysine on the one chromatogram, but the disadvantage at present of a three day reaction period to transform lysine residues to homoarginine.

(c) *Microbiological procedures.* The use of proteolytic microorganisms with exogenous requirements for essential amino acids has been intensively investigated in this country in recent years. Early studies were concerned with the assessment of overall protein quality (Stott, Smith & Rosen, 1963; Ford, 1960), but attention has now been turned to the measurement of individual 'available' amino acid contents.

Initially the protozoan *Tetrahymena pyriformis* was used. This method has the disadvantage that counting the number of protozoa is the most satisfactory method of measuring its growth. Ford (1962) developed a technique of partial enzymic hydrolysis followed by incubation with the proteolytic *Streptococcus zymogenes*, the growth of which can be measured turbidimetrically or from acid production. *Strep. zymogenes* requires seven amino acids including methionine and

tryptophan, but unlike *Tetrahymena* has no requirement for lysine. Neither microorganism has a sufficient need for cystine to be suitable for assay purposes.

Several laboratories have now successfully used the *Strep. zymogenes* procedure, particularly for the estimation of 'available' methionine. The method allows handling a large number of samples in a relatively short time, and the reproducibility is as good as that obtained with other conventional microbiological assays.

Results obtained by such procedures can only be considered appropriate to the practical problem of feed evaluation if they are comparable to results obtained by bioassays with the higher animals. Comparison of available methionine of animal proteins determined by *Strep. zymogenes* with the results of bioassays with the chick have shown that the original procedure, although ranking the materials in the correct order, significantly underestimated the chick value. Increasing the concentration of papain in the preliminary digestion resulted in values which could be equated directly to the chicks bioassay values (Miller, Carpenter, Morgan & Boyne, 1965). Many instances have been noted where the 'available' content has equalled the total. This marks a significant advance over other methods involving enzymic digestion followed by total amino acid analysis since in the latter only a small proportion of the amino acids are released, even in good-quality materials; hence results are comparative and can only be made semi-quantitative by comparison with a reference protein (Mauron, Mottu, Bujard & Egli, 1955; Ascarelli & Gestetner, 1962).

Application of the *Strep. zymogenes* technique to carbohydrate-containing materials also presents some analytical difficulties and the results obtained with this type of material have still to be checked against bioassay results.

### *Interrelationships between Available Levels of Amino Acids*

Lysine has frequently been considered to be the most labile of the amino acids. This is certainly true under conditions of mild heating of mixtures of protein and reducing sugar when the available lysine may be lessened without change in available methionine. On the other hand, heating proteins containing very little carbohydrates results in similar losses of lysine, methionine and probably of other amino acids in similar proportions (Miller, Carpenter & Milner, 1965).

With commercial fish and meat meals the FDNB-available lysine has been shown to correlate with the nutritive value of the protein when fed as the sole source of nitrogen to rats (National Research Council, 1963) and chicks (Kellenbarger, 1961). However, under these conditions of assay the sulphur amino acids are usually limiting and, thus, there appears to be a relationship between the available lysine and available sulphur amino acid contents. More directly

Miller, Carpenter and Milner (1965) have found the FDNB-available lysine to correlate with available methionine determined by chick bioassay. Ford (1962) and Waterworth (1964) have also reported correlations to exist between the available contents of several amino acids measured with *Strep. zymogenes*. These correlations found with commercial meat and fish meals are due mainly to muscle being higher than connective tissue in both lysine and methionine so that variations in the proportions of muscle and connective tissue affect both amino acids similarly, and possibly, in addition, to heat damage reducing the availability of several amino acids.

Thus for animal protein concentrates containing little carbohydrate, either FDNB-available lysine or *Strep. zymogenes* available methionine indicates the value of the protein as a source of these two amino acids and probably indicates the availability of other amino acids as well. Use has been made of this relationship to correct total amino acid analysis by a determined availability factor. Both Donoso, Lewis, Miller and Payne (1962) and Mason and Weidner (1964) have found that nutritive values calculated by including both FDNB-available lysine and total amino acid values correlated more closely with the biologically determined values than predictions based on total amino acid analysis alone.

Specific examples of incomplete availability of amino acids in protein foods, and the effects of heat in reducing availability have been quoted. How often do such samples or damaging conditions arise in normal commercial practice? As yet there are insufficient results from bioassays to answer the question. The FDNB-available lysine values reported by Pritchard *et al.* (1964) showed that while 50% of fish meals come to within $\pm 10\%$ of the mean value, the other 50% were distributed between very wide limits. The application of techniques of measuring available amino acids to control the selection of raw materials and processing conditions could lead to considerable improvements and standardisation of quality.

There are even less data for the availability of amino acids in oil seeds and cereals. Lysine and methionine both appear to be poorly available (approximately 70%) in raw soya beans or isolated soya bean protein, but values for properly heated meals have ranged from 80 to 120% and can be taken to indicate nearly complete availability (Guthneck, Bennett & Schweigert, 1953; Ousterhout, Grau & Lundholm, 1959; Kwong, Barnes & Fiala, 1962; Guttridge & Lewis, 1964). The availability of methionine appears to be somewhat lower in groundnut meals, with values in the range 70–88% (Guttridge & Lewis, 1964).

Gupta, Dakroury, Harper and Elvehjem (1958) report lysine availability values for the rat in maize, wheat flour and rice of approximately 50, 70 and 90% respectively. Calhoun, Hepburn and Bradley (1960) also report values in the range 70–80% for availability of lysine

in wheat and wheat flour. In contrast to the low availability, the lysine of cereals is approximately 90% digestible (Kuiken & Lyman, 1948; de Muelenaere & Feldman, 1960). Clearly there is a need for further biological assays to confirm these low availability values. There is also a need to develop laboratory tests such as the FDNB reaction and *Strep. zymogenes* assay for the assessment of available amino acids in this type of feed.

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# 2

# METHODS FOR MEASURING PROTEIN QUALITY WITH CHICKS

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*Synopsis*

Feed protein quality can be determined in various ways, depending on the viewpoint of the person measuring it. The producer measures it by feed conversion efficiency or in economic terms. The nutritionist measures it by much more precise methods of the kind described and discussed in this paper. All these methods depend on numerous factors, among which the feed protein level is the best understood. Other factors, such as the growth rate of the bird and the energy content of the feed, have been explored less fully and receive special consideration in this paper.

Of all the measures proposed, Protein Efficiency Ratio is the last to be commended. Gross Protein Value is more reliable, as measured under feeding conditions nearer to those of practice. The most exact measures depend on estimation of nitrogen retention. Among them, Biological Value and Maximum Daily Protein Anabolism give perhaps the best measures of protein value, though their meanings are not equivalent. They have the advantage of constancy over a given range of feed protein levels. This advantage is not shared by Net Protein Value which is based on body analysis; several findings, indeed, emphasise the variation in chick body composition with the amount of nitrogen ingested. A method of feeding is proposed whereby this disadvantage can be alleviated.

Unfortunately, the experimental conditions appropriate to the various measures of feed protein value differ from those which obtain in commercial poultry houses, and therefore from those in which practical diets are consumed.

## *Introduction*

INCREASING knowledge of the physiology of nutrition has led to considerable advances in animal feeding, and in particular has made it possible to define more precisely the nutritional requirements of each species of domestic animal. Experimental data, fed into electronic computers, form the basis of dietary formulation. But the validity of the computer formulation depends entirely on the validity of the data fed in, and among the most important of these—as well as the most difficult to

define—is what the practical feed compounder calls the 'quality' of the ingredients.

'Quality' has numerous aspects that are difficult to define and even more difficult to evaluate. As used with reference to protein feedstuffs for birds, the 'quality' of a feed represents, to the producer, its ability to ensure body maintenance and to support growth or egg production in his birds. He measures it by the *Feed Conversion Ratio*. To the consumer, the 'quality' of feed given to the chicken is something quite different, because it takes no account of feed efficiency. He judges the quality of the feed through the quality of the product: conformation of the carcass, cooking characteristics, taste and sanitary quality. Finally, to the nutritionist the term 'quality' has scarcely any meaning at all, because it is the sum total of many criteria of efficacy. Most important among these is protein efficiency, i.e. the efficiency with which the feed proteins can be converted into tissue proteins. This transformation clearly depends on the composition of the proteins *sensu strictu*, but it also depends on a series of other factors such as the physiological status of the animal, its environment, and the composition of the feed. It depends also on the way in which the various proteins are associated in the diet, and on the non-protein components of the feed. Thus, as Munro (1964) has said, '. . . individual nutrients are not consumed in isolation but as a part of a diet providing a large number of variable components', and the feed value of a protein does not depend solely on its own characteristics.

This last point is extremely important. One must make a distinction between *protein* and the *protein component of a feed*, because one cannot, in a feed, deal with the proteins in isolation from the non-protein components. The nature of the ternary constituents, the mineral salts and the vitamins in the same ingredient, may modify its protein efficiency. So may contaminants polluting the pellets or crumbs, the cereals and the meat- or fish-meal components of the feed.

All these factors, which the nutritionist tries to disentangle, together determine the overall 'quality' of the feed. Thus 'quality', which producers recognize through their sensory perceptions, represents the overall resultant of a whole series of criteria that is very difficult to measure and feed into the computer.

One must nevertheless do so, because 'quality' is the principal nutritional factor limiting the performance of feed. Combs, Quilin and Helbacka (1958) have shown that one could raise chickens, and even obtain nearly ideal performance (feed efficiency=1·01), on semi-synthetic diet in which all the nutrients were quantitatively defined. One cannot, unfortunately, reproduce such performance in the commercial poultry house, by reason of cost, and one must be content with natural feedstuffs. Thus the problem is to determine the biological value of natural feeds and feed ingredients in the same manner that the biological value of synthetic nutrients is known. Today, when the

producer price for broilers is extremely low and the demands of the consumer are increasingly exacting, the problems of feed formulation and feed utilization by the birds are crucial. In France, the producer must obtain a feed conversion ratio better than 2·1 to 1 if he is not to lose money on a crop of poussins. This shows how important it is to develop precise definitions of the 'quality' of feed ingredients and to provide compounders with methods for estimating it.

To resolve this problem, the theoretician must turn to fundamental studies of nitrogen metabolism, which originated with Magendie (1816) and were continued by Boussingault (1851). The latter showed that one nitrogenous nutrient can be substituted for another provided that due attention is paid to the level of incorporation in the diet. He expressed the nutritional value of a nitrogenous feedstuff as the percentage of that of meat, and thereby laid down the first biological method for the evaluation of quality. Since that date numerous investigations have been undertaken in this field, the majority of them using the dog or the rat. The findings are well known and have been reviewed recently by Allison (1955), by Adrian and Rerat (1958) and in the report of the Symposium held in 1957 at the Royal Free Hospital School of Medicine. Mitchell (1964) has given details of more recent work using these methods. There have been far fewer fundamental studies of nitrogen metabolism in the bird, where separation of faeces and urine can be achieved only by means of a delicate surgical operation. Nevertheless, the chicken has been fairly widely used in experiments of an applied nature that are of special interest to us. We shall not recapitulate the principles of measuring protein value, but we shall instead discuss the conditions under which they may be applied and the causes of variation in the results.

### *Review of Overall Methods of measuring Protein Quality*

(a) *The weight method*

The simplest method consists of measuring the weight gain of young chickens. However, comparison of proteins by this method is not possible unless the protein content of the feed is accurately defined. Fig. 1 shows, for example, the variation in weight gain of 5-week-old Rhode × Wyandotte chickens as a function of the protein level in the feed. For each protein there is a characteristic shape of the rising part of the curve, a characteristic maximum weight gain and the corresponding value of the optimum dietary protein level. This method is not valid unless one limits the quantity of feed, and therefore of protein, ingested. With *ad libitum* feeding one can measure protein value only by relating weight gain to the amount of protein ingested; this is the Protein Efficiency Ratio (PER) of Osborne, Mendel and Ferry (1919). This method has been criticized severely on the ground that it does not incorporate a precise definition of either of the items to be compared.

It was for this reason that Heiman, Carver and Cook (1939) introduced the method whereby chickens receiving a feed incorporating the protein to be tested were compared with others receiving a reference protein. This method entails graphic interpolation. It is the Gross Protein Value method and it expressed the value of a protein as a percentage of the value of a reference protein, just as Boussingault had done a century earlier. It was improved by Carpenter, Ellinger and Shrimpton (1955), who defined the levels of cellulose, minerals and vitamins in the reference diet.

One may ask if the overall values yielded by these methods give sufficient information for computer formulation. Feed proteins are to be considered, above all, as sources of amino acids. An essential feature of the measurement of 'quality' is therefore to discover the amino acid composition of the proteins and compare it with the requirements of the animal, as listed by Almquist (1947) and more recently by Combs and Nicholson (1962). The greater the quantity of protein needed to satisfy the amino acid requirements, the worse the quality of the

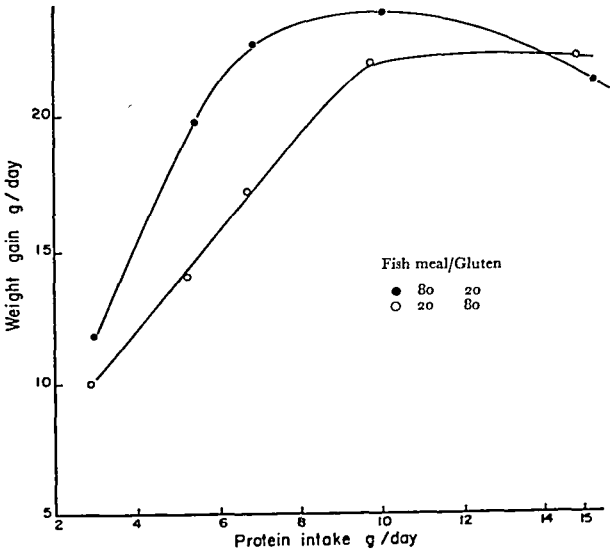


FIG. 1. Variation in weight gain by chickens receiving increasing amounts of mixtures of fish meal and gluten.

protein. It was following this line of argument that Mitchell and Block (1946) defined their 'chemical score', and Oser (1951) his index of essential amino acids. However, it is not sufficient that the amino acids should be present in the feed and that they *should constitute* an appropriate proportion of it; they must also be available. This is why the majority of chemical measuring procedures, of which the best known is that of Carpenter and Ellinger (1955), are based on the availability of the amino acids. *In vivo*, attempts have been made to relate the growth of the chick to the methionine content of the feed and this was one of the first methods of bioassay of sulphur amino acids (Grau and Almquist, 1943, and more recently Miller and Donoso, 1963). Another method of measuring the availability of protein amino acids *in vivo* has been proposed by Ousterhout, Grau and Lundholm (1959) and it too rests on the weight gain of chicks. The birds are given a diet in which 10% of the protein is supplied by the test substance and which is enriched with all the essential amino acids—except the one whose availability is under investigation—supplied in pure, crystalline form and in quantities sufficient to meet the bird's requirements. The amino acids are investigated one by one. Comparison of the weight gains obtained under the different regimes and with a complete diet enables one to establish the order in which the amino acids become limiting. This technique is very accurate but it is time-consuming and very expensive. Furthermore, in practice it is not necessary to know the availability of all the amino acids in a protein; one need know only that of the more important ones. It was following this line of thought that Davidson and Boyne (1962) studied the availability of methionine and lysine in peanut meal. It must be remarked, however, that Laksesvela (1958) is not completely satisfied with this technique.

Interpretation of the results of amino acid analyses of proteins demands comparison with those from a reference protein. But the choice of reference protein, which has been discussed by Jacquot and Vigneron (1958), is not simple; there is no reference protein that is appropriate in every case. These methods can also be criticized on a second ground: knowledge of the chemical composition of the protein constituents of a feed is not, alone, sufficient to enable one in practice to define the 'quality' of the feed. One may cite as an example animal proteins* that are accompanied by vitamin $B_{12}$; its role in nitrogen metabolism is well known (Calet, 1954; Calet, Rerat & Jacquot, 1954; Henry and Kon, 1956).

(b) *Nitrogen-balance and whole-body-analysis methods*

The only satisfactory way of finding the rate of transformation of feed protein into body nitrogen is to measure it directly. For this

* Terroine considers it wrong to speak of 'animal proteins'; he prefers 'protein of animal origin'.

purpose one uses the balance-sheet method and the body-nitrogen-analysis method (known less elegantly as 'the carcass method').

The former method yields the components of the efficiency of nitrogen metabolism: Digestibility and Biological Value. In birds a prerequisite is colostomy, a delicate surgical operation to form an artificial anus (Ariyoshi & Morimoto, 1956; O'Dell, Woods, Laerdall, Jeffray & Savage, 1960; Hartfiel, 1962; and, more recently, Tasaki & Okumura, 1964, and Squance and Brown, 1965).

One can estimate nitrogen retention from an overall balance sheet without distinguishing the two sources of the nitrogen in the droppings. This yields the *coefficient d'utilisation pratique* of Terroine and Valla (1933), which relates the nitrogen retained to the nitrogen ingested. This is the method used by Van Landingham, Clark and Schneider (1942).

The second way of measuring nitrogen retention is based on estimation of the difference in body nitrogen between the beginning and the end of the experiment. The initial body nitrogen is estimated from its regression on body weight ($r=+0{\cdot}62$) or, better, from its rate of gain in weight before the experiment ($r=+0{\cdot}75$) (J. Guillaume, unpublished). This estimate has the advantage that it takes account both of the absolute weight of each experimental animal and of its growth rate. Although the error of estimation may be small in relation to the nitrogen retained, because the young animal at the beginning of the experiments represents only a small amount of nitrogen, nevertheless, as we shall see later, the accuracy of the estimate of initial body nitrogen is open to criticism.

It is for this reason that Bender and Miller (1953*a*) and Miller and Bender (1955) have proposed a measure of protein efficiency called Net Protein Value (NPV), which is the product of the Biological Value and the Digestibility. Calculation shows that it depends on the difference between the amounts of nitrogen in an experimental animal receiving the protein under test and one receiving nitrogen-free feed. One can therefore estimate it from body analysis data and one speaks of Net Protein Utilization (NPU). Two dosage levels are then all that is required, and the long and tedious determinations of endogenous faecal and metabolic nitrogen are avoided. It gives an overall picture of the factors affecting protein value. However, if one is to use the method proposed by Bender and Miller (1953*a*), one must first confirm that body-analysis and nitrogen-balance methods lead to the same result. Harnisch and Becker (1958) have shown that in the fowl and in the pig the results given by the nitrogen-balance and body-analysis methods agree closely, provided that one takes care to avoid losses in the collection of droppings, by means of metabolism cages. Butterworth (1962) considers that in practice the methods are equally satisfactory, and Ivorec-Szylit and Calet (1964) take the view that one can estimate nitrogen retention by either method in the laying hen because

the difference between results obtained by the two methods is small and constant from bird to bird, provided that the environment is properly controlled.

The method proposed by Bender and Miller has been used successfully in the growing chicken by De Mueleneare, Quicke and Wessels (1960*a*) and by Summers and Fisher (1961*a* and *b*).

Among the modifications to this method that have been proposed, some are minor (Bender & Doell, 1957*a*) and others are important. The latter rest on the supposition that the body composition of the growing chicken can be considered to be constant, provided that the bird is young and the duration of the experiment is short. Thus Bender and Doell (1957*b*) replace body nitrogen with liveweight in their calculations. They then speak of Net Protein Retention (NPR) or of Protein Retention Efficiency, which is the NPR expressed as a percentage. The second modification consists of replacing body nitrogen by body water in the calculations, because in a group of 150 rats aged from 33 to 57 days the ratio N to $H_2O$ was independent of age (Bender & Miller, 1953*b*). The simplification due to these modifications is evident and the method has been used to evaluate numerous feedstuffs in the fowl by Ascarelli and Gestetner (1962), Fisher, Summers, Wessels and Shapiro (1962) and Summers and Fisher (1962).

One criticism of the NPU or NPR method is that the level of daily feed intake is not fixed. Furthermore, the intake is highly variable with feed regime, and especially in the case of the nitrogen-free regime. It was to meet this criticism that Rand, Collins, Varner and Mosser (1960), following the Gross Protein Value method, allowed their birds to feed *ad libitum* but related their weight gains to the quantity ingested. One can thus compare, by interpolation, the weight gains of animals that have consumed the same quantity of feed and of nitrogen. As in the method of Heiman, Carver and Cook (1939), the results are published as a percentage of a reference protein, isolated Soya bean protein (Drackett Assay Protein C-1). The importance of variation in the amount of feed consumed has not escaped the notice of the protagonists of the NPV method, who have proposed a further criterion called the Net Dietary Protein Value (NDPV) and subsequently the Net Dietary Protein Calories Percentage (NDPCals %), which is a product, NPV × dietary protein level, where this level is expressed either in grams or in calories (protein calories as a percentage of total calories) (Platt and Miller, 1959). We may note here that this concept, which takes account of nitrogen utilization as a function of ingested energy, has the same significance as the *production quotient* of Möellgaard (1929) when applied to growth.

The estimation of NPU by means of body water measurements and the significance of NPR call for some comments.

1. Not all the workers who have used the rat as their experimental animal are agreed that one can estimate body nitrogen from a measure-

ment of body water. On the one hand Bender and Miller (1953*b*), Miller and Bender (1955) and Bender and Doell (1957*b*) reported that the ratio N to $H_2O$ is constant, even in the presence of appreciable variation in the body lipid content. This is in agreement with Behnke, Osserman and Welham (1953), who reported that the composition of the lean body mass is constant in all adults. On the other hand, Henry and Toothill (1962) do not find the same regression of the N to $H_2O$ ratio on age of animal in all circumstances. It differs between males and females. Furthermore, it varies according as the rats receive a feed that contains or does not contain protein. This is particularly important, because one measures the protein value of a feed by reference to one that is protein-free. Rerat, Fevrier, Henry and Lougnon (1964) found that the ratio rises with liveweight, but not in a linear manner. Similar disagreements are found among those who work with the chicken. According to De Muelenaere *et al.* (1960*a*), chickens fed a 7% protein diet have a body $H_2O$ to N ratio that is constant. Likewise according to Summers and Fisher (1961*a*) the ratio was constant and equal to 24·7 when the feed protein level varied from 0 to 26%. It may have been slightly higher in birds on the nitrogen-free diet, but the difference was not statistically significant. The ratio held constant whether the birds were fed a practical or a purified diet. However, the data of Hunt (1965) do not agree with those in the earlier reports. According to him, the N to $H_2O$ ratio increased with age of the bird (between 7 and 29 days) and the regression line varied significantly with the feed protein level. With 22% of protein in the feed the birds had relatively more nitrogen than those

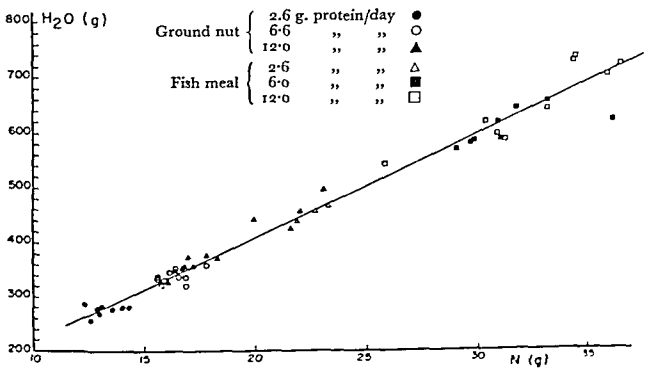


FIGURE 2. Relation between body water and body nitrogen in chickens aged 7 weeks receiving three levels of protein.

fed on a 15% protein diet. Finally, we may note that there were interactions between strain, sex and age, and that the results differed from hatch to hatch. Similarly Fraps and Carlyle (1941) reported that the nitrogen content of the lean weight gain varied from 20 to 27 % according to the weight of the animal. Furthermore, results from our laboratory by P. Delpech and J. Guillaume (unpublished) do not confirm the constancy of the N to $H_2O$ ratio of the body of the chicken. Fig. 2 shows the relationship between body water and body nitrogen in chickens of the same age (7 weeks) grown in individual cages and fed on 6 different diets (3 nitrogen levels and 2 protein sources). One sees, as the authors described, an extremely close correlation between the two series of values. The regression equation is

$$\text{Water} = 27{\cdot}7 + 19{\cdot}208\text{N}$$

It follows that the ratio N to $H_2O$ cannot be constant, because the regression line does not pass through the origin. One can derive the equation relating the N to $H_2O$ ratio to the total body nitrogen; it is the hyperbola

$$\frac{\text{N}}{\text{Water}} = \frac{\text{N}}{27{\cdot}7 + 19{\cdot}208\text{N}}$$

Fig. 3 shows that it is indeed so, and confirms the results of Rerat *et al.* (1964), who worked with the rat. Thus the animals contain relatively more nitrogen than water when their growth rate is greatest. This conclusion is reinforced by a more detailed examination of Fig. 3. In it one can distinguish each of the experimental groups and one finds

TABLE 1

*Regression of body water content on body protein content of chickens under various feed regimes*

| Treatment | Number of observations | Regression equation* | Correlation coefficient |
|---|---|---|---|
| Control | 19 | $y = 3{\cdot}81\,x + 12{\cdot}6$ | +0·93 |
| Starvation for 3 days | 19 | $y = 3{\cdot}44\,x + 9{\cdot}2$ | +0·96 |
| Weight held constant for | | | |
| 3 days | 20 | $y = 3{\cdot}76\,x + 5{\cdot}2$ | +0·98 |
| 6 days | 20 | $y = 3{\cdot}18\,x + 18{\cdot}1$ | +0·98 |
| 9 days | 20 | $y = 3{\cdot}32\,x + 14{\cdot}6$ | +1·00 |

*$y$ = amount of body water.
$x$ = amount of body protein ($N \times 6{\cdot}25$).

that the regressions differ significantly with treatment when the growth rate is low.

One further observation confirms this. Fig. 4 shows the variation in body water as a function of body protein in chickens that have been subjected to starvation for 3 days or have been held at constant weight for 3, 6 or 9 days by feed restriction; one group, of control birds, has

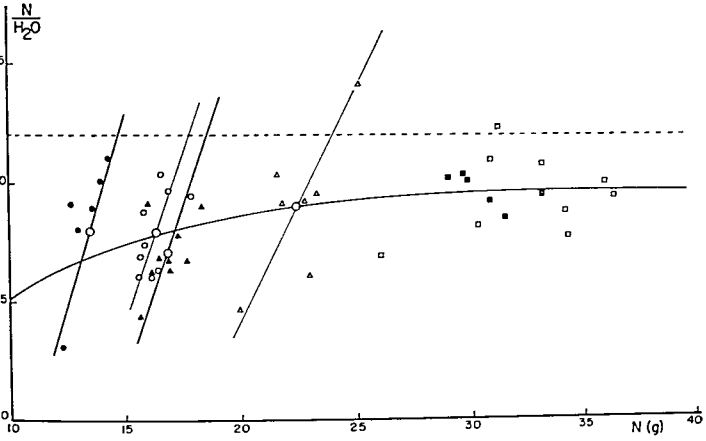


FIG. 3. Variation of $N/H_2O$ and body nitrogen (for details see fig. 2).

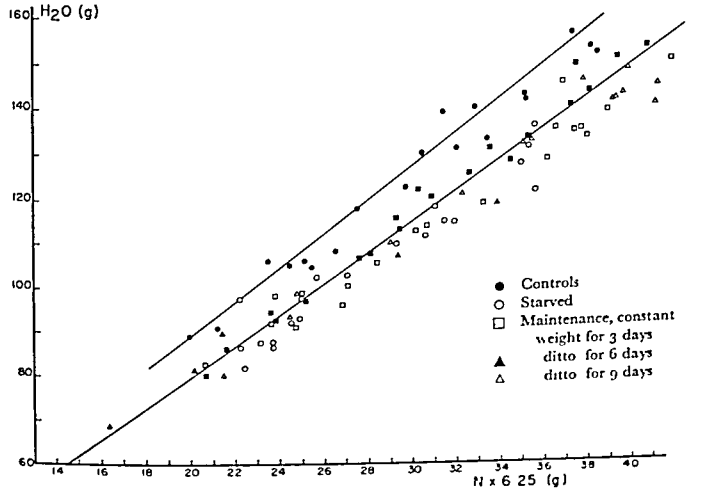


FIG. 4. Relation between body water and body protein in 4 weeks old chickens under different experimental conditions.

not been subjected to any special treatment. Here again the regressions are excellent, as one can see from the correlation and regression coefficients in Table 1. It is seen that the regression lines in the experimental groups are parallel and that they differ significantly from that in the control group. The regression equations are:

| | |
|---|---|
| Control | $\text{Water} = 12{\cdot}6 + 3{\cdot}81\text{N} \times 6{\cdot}25$ |
| Experimentals | $\text{Water} = 12{\cdot}2 + 3{\cdot}41\text{N} \times 6{\cdot}25$ |

None of the lines passes through the origin and so the ratio N to $H_2O$ cannot be constant.

These results, taken together, show the bearing that growth rate has on the method proposed by Bender and Miller (1953*a*, *b*). One sees that all the factors that affect growth rate, such as age, strain and sex, affect the accuracy of the method. This is because they affect protein efficacy, which is the very thing we are trying to measure. To overcome this difficulty, it is necessary to consider not the means of the observations, as Miller and Bender (1955) recommend, but the individual results through regression analysis.

2. One can similarly discuss the interpretation of NPR, or better of PER, and compare them with NPU. Several authors have reported good concordance between the two series of values. The coefficient of correlation found with five different proteins, enriched or not with amino acids, was 0·9 according to Summers and Fisher (1961*a*); a similar conclusion was reached by De Muelenaere, Quicke and Wessels (1960*b*). This implies that the composition of the protein element in the weight gain of the chicken is constant, whatever the nutritional regime. Similarly De Muelenaere, de Martin and Murdoch (1965) studied feed regimes in which the protein content varied from 7·5 to 15%, and failed to find significant differences between the regressions relating their NPU and NPR values; the mean slope of the regression lines was 15·5. These results are not universally accepted. In the first place, one finds in the literature values for the NPV to NPR ratio that vary from 15 to 18, depending on the experiment and the worker. Furthermore, it is known from the work of Donaldson, Combs and Romoser (1956) that when the protein level varied from 27·45 to 17·10% in feeds with 970 productive cal/lb, the body protein content fell from 20·3 to 18·3%. Similar conclusions were reached by Yoshida, Hizikuro, Hoshii and Morimoto (1962).

Our own results confirm that the body composition of the chicken depends both on the quantity and on the nature of the proteins ingested. Table 2 shows the results of an experiment in which 7-week-old chickens were put on two nutritional planes. In one of them the protein was supplied in limited quantity independently of a protein-free feed provided *ad libitum* following a technique described by Calet and Melot (1961). In the other the birds received the same amount of energy in the form of a complete mixed diet that was limited in quantity. Two

different proteins were provided at 2·6, 6·5 and 12·0 g per bird per day. Table 2 shows the variation in weight and in body protein content. The influence of the amount of protein ingested on the body protein content is evident. Furthermore, it was more marked when the nutritional regime was most closely defined, i.e. when the feed was mixed and limited in quantity. It is also seen that the most effective feed proteins correspond with the highest body protein contents. One may conclude from the mean values that the body protein level increases

TABLE 2

*Effect of the quantity, nature and distribution of feed proteins on the body composition of the bird*

| | Feed protein (gram/day) | Liveweight (g) | Body protein (N × 6·25)(%) |
|---|---|---|---|
| Mixed feeding | | | |
| Fish meal | 2·5 | 357 | 19·01 |
| | 6·3 | 476 | 20·54 |
| | 11·8 | 530 | 21·09 |
| Peanut meal | 2·6 | 316 | 17·68 |
| | 6·4 | 407 | 19·16 |
| | 12·0 | 487 | 20·40 |
| Separate feeding | | | |
| Fish meal | 2·6 | 390 | 18·03 |
| | 6·6 | 552 | 18·67 |
| | 11·8 | 679 | 21·19 |
| Peanut meal | 2·7 | 301 | 19·50 |
| | 6·7 | 404 | 19·00 |
| | 12·0 | 539 | 19·30 |

each time nitrogen retention is improved in absolute value. Consideration of the individual values is still more enlightening. Fig. 5 shows the variation in body nitrogen content of other 7-week-old birds as a function of their weight gains. The birds, housed in individual cages, received two proteins of different value, distributed at different levels. The feed also contained lipids or glucose. One sees clearly the effect of the quantity of protein ingested, which was 0, 3·1 or 12 g/day. One is also struck by the regressions of the body nitrogen content on growth rate, but even more by the fact that for each nutritional regime there is a negative correlation between body nitrogen content and growth rate. In the groups on protein-free feed the regression is identical with that in the groups on feed containing oil or sugar. At the lowest nitrogen levels the regressions differ significantly according to the source of the protein. At higher nitrogen levels the correlation is less good. Be that as it may, these results demonstrate that the body nitrogen content of the chicken depends not only on the nature and amount of protein ingested, but with a given protein it depends also on the growth rate of the animal; the faster the chicken's growth, the less the body nitrogen proportion. These results should not surprise us.

It is known from the work of Wilson (1954), of Widdowson and McCance (1960) and of Dickerson and Widdowson (1960) that the various tissues do not develop with the same rhythm when there is variation in nutritional plane or growth rate. It is known, for example, that plumage and the skeleton are tissues of high priority and that their development proceeds even during periods when the feed intake is minimal and the animal is not increasing in weight. The same conclusion was reached by Davidson, Mathieson and Williams (1962) from the study of the composition of weight gains of chickens fed only on cereals and showing, as a result, low growth rates.

(c) *Indirect methods of measuring nitrogen retention and Biological Value*

1. *Hohls's method.* Since 1955 Hohls has been concerned with the problem of determining the Biological Value of proteins and its application to birds. He argues as follows (Hohls, 1958*a*). Among birds of a given weight, the protein content of the gain in fat-free weight is constant; it is about 25% in chickens of 1 kg. If he knows the quantity of lipid accumulated in the body of a growing bird, he can then deduce the nitrogen retention. By means of respiratory exchange measurements and weight gain, he then measures the net energy value of growth. He can find the composition of the weight gained and the

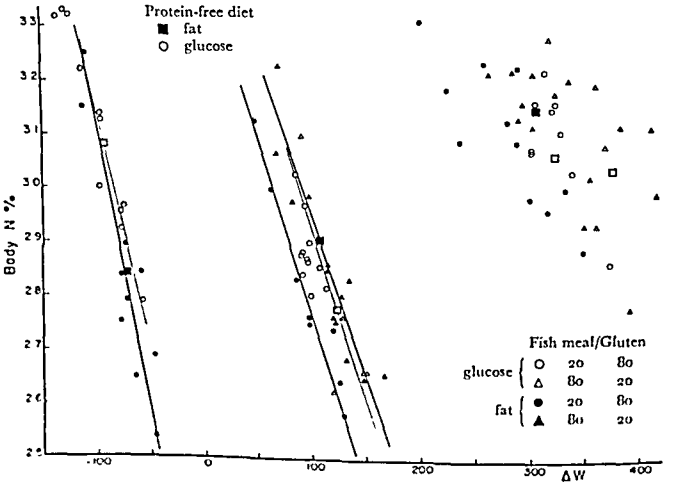


FIG. 5. Regression of body nitrogen content on weight gain in chickens receiving 0 or 3 and 12g. protein per day.

quantity of protein deposited. The error of the estimates is about 5%, i.e. about the same as that with the nitrogen-balance method. But Hohls can go further in his calculations: by estimating nitrogen digestibility with the aid of coefficients, he can estimate the excreted urinary nitrogen. In order to allow for maintenance losses, all the values obtained are related to the metabolic parameter, $W^{2/3}$. He has studied variation in urinary nitrogen as a function of numerous factors. Though the method employed may be surprising, Hohls's results are in full agreement with those of Allison and Anderson (1945) obtained with the direct method in the dog. We reproduce in Fig. 6 the two graphs in which Hohls (1955*b*) relates variations in urinary nitrogen and Biological Value to the digestible nitrogen. The first graph falls into two parts: one in which the slope is equal to 1—BV/100, as Allison has shown; the other is parallel to the first bisector (45° diagonal), indicating that the balance cannot increase any further.* Barnes,

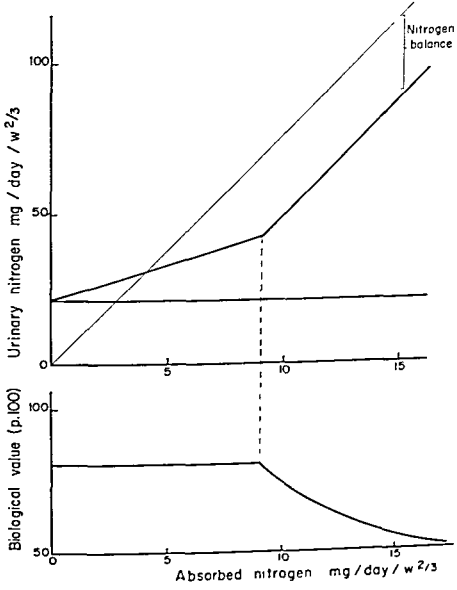


FIG. 6. Urinary nitrogen excretion and Biological Value of proteins in relation to absorbed nitrogen (Definition of "maximal mögliche tägliche Eiweissansatz" after Hohls, 1955, by permission).

* The balance is the difference in the ordinate between the curve and the first bisector.

increases when the Biological Value is constant. The two criteria do not have the same significance; the first expresses the efficacy of the protein, while the other expresses the maximum potential of the protein for conversion into tissue. Far from being contradictory, the two criteria complement one another.

2. *Arnould's method.* The approach adopted by Arnould (1961) gives a new concept of nitrogen metabolism. He observes that all the classical theories neglect the influence of nitrogen level on the intake of the animal. But Arnould (1961) and Calet, Jouandet and Baratou (1961) simultaneously demonstrated the considerable influence exercised by the quantity and quality of the proteins on the amount of the ingesta. The amount of food consumed per unit weight decreases with the feed protein level. This phenomenon is seen in the rat as well as the chicken. Arnould, however, distinguishes that part of the feed which is used for growth from that part which is used for maintenance and estimates the amount of feed nitrogen which is converted into tissue nitrogen. By allowing for the part of the feed used for body maintenance, Arnould provides an explanation for the majority of the discrepancies from the classical theory encountered in the course of experimentation. The interest of Arnould's theory for us stems from the following observation. For a given energy level the amount of feed consumed per unit of body weight is related to the growth rate of the

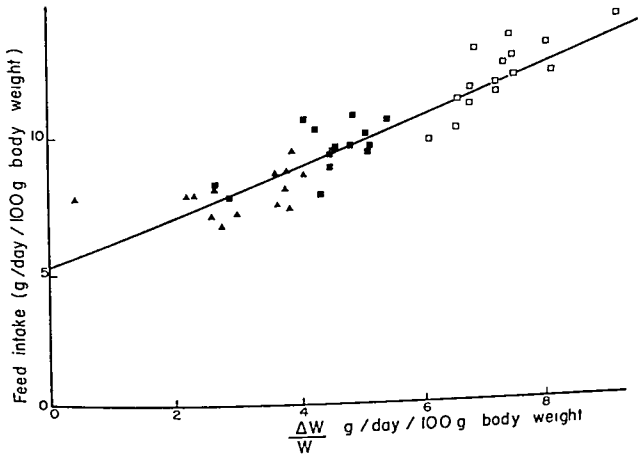


FIG. 8. Relation between feed intake per unit live body weight and rate of growth of chickens aged ▲ 4 weeks, ■ 5 weeks and □ 6 weeks. (Definition of feed conversion for growth after Arnould, 1961.)

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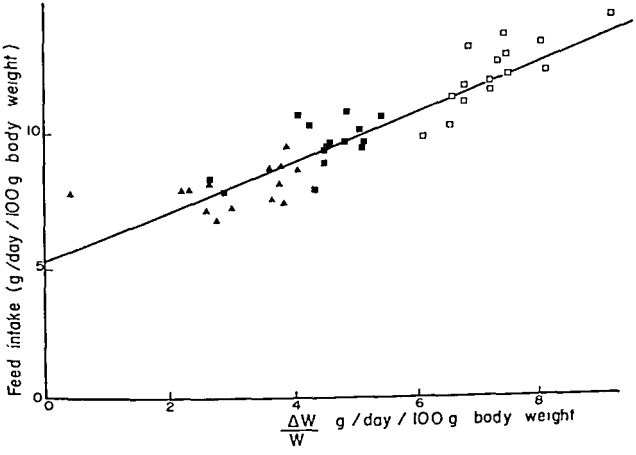


FIG. 8. Relation between feed intake per unit live body weight and rate of growth of chickens aged ▲ 4 weeks, ■ 5 weeks and □ 6 weeks. (Definition of feed conversion for growth after Arnould, 1961.)

animal by the regression relationship reproduced in Fig. 8. One observes first that the ordinate at the origin is constant, independent of the growth rate, the feed protein level and the nature of the feed proteins. Arnould thus defines a constant maintenance energy requirement. Next, one observes that the slope $b$ of the regression* represents the rate of conversion of feed for growth only. This coefficient varies according to an inverse function of the feed protein level and the value of the protein. Arnould verified experimentally that the curve

$$b = f(P - P_0)\dagger.$$

is a hyperbola. It follows that the product $b(P-P_0)$ is constant, independent of feed protein level, the growth rate of the animals, and their age. Let us suppose that the part of the feed nitrogen required for growth is totally utilized (i.e. the case of a protein with B.V. equal to 100), then the product $b(P-P_0)$ represents the quantity of protein deposited in the body per unit weight gain. Experimentally, the product $b$ is found to be 0·16 in the rat, a value which corresponds precisely to the body protein content, 16%. When one is dealing with feed proteins of different Biological Values, one has:

$$b_1(P-P_1) \times BV_1 = b_2(P-P_2) \times BV_2 = b_3(P-P_3) \times BV_3 = 16\%$$

Theoretically one can then estimate the Biological Value by a simple method the results of which are independent of the protein level in the feed used to measure it.

TABLE 3

*Values of the coefficient b and maintenance requirements of the chicken, determined by Arnould's (1961) method*

| Feed protein level (%) | $b$ | Maintenance (kcal/100 g liveweight) | Correlation coefficient† |
|---|---|---|---|
| 6 | 1·99 | 26·2* | +0·62 |
| 10 | 1·59 | 21·4* | +0·79 |
| 15 | 1·44 | 14·1 | +0·91 |
| 21 | 1·27 | 13·3 | +0·90 |
| 29 | 1·01 | 15·0 | +0·93 |
| 38 | 0·98 | 15·9 | +0·91 |

* The weak correlation between the amount ingested and the weight gain makes the estimation of the maintenance requirement imprecise.

† Based on 15 observations.

We have tried to test the validity of this method in the chicken, Guillaume (1966) has confirmed that the value of the maintenance requirement is constant, as the values in Table 3 show. The constancy of the product $b(P-P_0) \times BV$ is open to further discussion because the

* $b = (I-A)/\Delta W$, where $I$ is total food intake, $A$ is the quantity of feed required for maintenance and $\Delta W$ is the weight gain.

† $P$ is the protein level of the various feeds; $P_0$ is the protein level of the feed that just meets the maintenance requirement (4% for egg, 5% for casein).

body composition of the bird varies as a function of the feed protein level. However, Arnould's method is open to the same criticisms that we have listed in relation to NPR. According to Arnould himself (personal communication), several improvements are necessary before the method can be used with birds, but one must recognise the interest in the theoretical aspects that he has brought to light.

### *Comparison of the Methods and Discussion of the Results*

Having described the various methods for measuring the 'quality' of proteins, we must test them and compare the results obtained under various experimental conditions.

#### (a) *The influence of factors inherent in the animal*

It has long been recognized that animals utilize their feed, and especially their nitrogen, less and less well as they age (Henry and Kon, 1957). With birds, it was Hohls (1955*a*) who first demonstrated the effect of age on the Biological Value of proteins. This is especially important after 10 weeks of age. Hohls (1959*a*, *b*) also demonstrated the effect of strain and sex on the ability of the bird to metabolize protein. Cornish fowl show a lower urinary-nitrogen-to-retained-nitrogen ratio than White Leghorns, and a higher maximum daily protein anabolism. It is the same with males vis-à-vis females. The maximum daily protein anabolism is a genetic characteristic of the animal. Furthermore, it has been put to use in the selection of strains (Boyer, de Laage and Calet, 1963). One may ask if this property may not be a consequence of the growth rate of the birds.

Fig. 9 shows the individual variation of NPU as a function of the weight gain of 7-week-old chickens. They had been used in a $2 \times 2 \times 2$ factorial experiment in which the variables were the quantity and nature of the proteins and the source of the ternary feedstuffs (oil or glucose). The latter were fed independently of the proteins.

When all the feed regimes are shown together on the graph, one sees a close correlation between NPU and weight gain. Only the effect of the quantity of nitrogen ingested appears clearly. For a given feed protein level one can find relationships between the other two variables, but they are not statistically significant. We shall have occasion to return to these results, but must emphasize once again the importance of growth rate to the interpretation of the results.

#### (b) *The influence of nutritional factors*

1. *The feed protein level* has a considerable effect on the value of the different measures. The spectacular variation of PER in the mouse, as a function of feed protein level, established by Barnes and Boshardt (1946), is now well known. A similar phenomenon occurs in the

chicken, as is shown by Fig. 10, in which the data are drawn from our own work. Although the variation is less marked, Gross Protein Values likewise vary with feed protein level. It can be seen from the data of Rand *et al.* (1960) that growth response is proportional to protein intake. This holds good because the animals were young and the variation in feed intake was small. Heiman, Carver and Cook (1939) reached the same conclusion where the amount of protein ingested was small. When it was large, the weight gain rose less rapidly than the protein intake and the GPV became variable. From the graphs shown by Heiman, Carver and Cook we have calculated the Gross Protein Value of Soya bean meal as a function of the ingested protein (Table 4). These results show that the differences between protein efficiencies decrease gradually at high feed protein levels. At

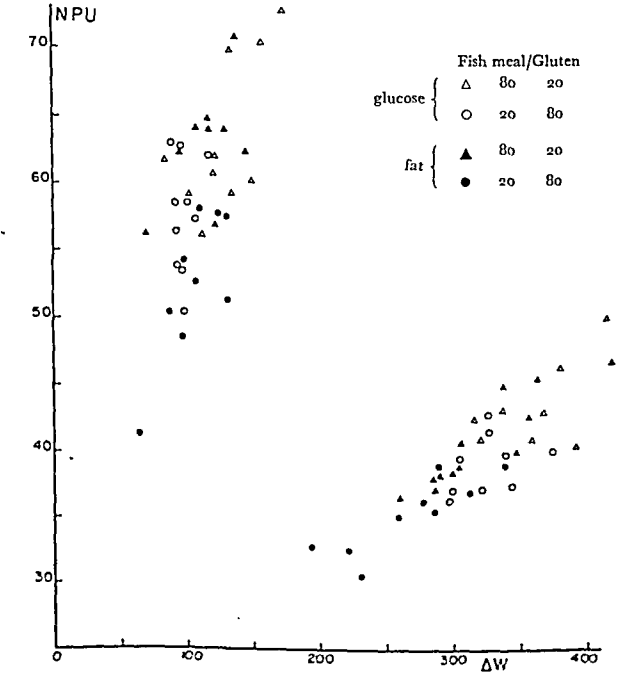


Fig. 9. Influence of rate of growth of chickens aged 7 weeks on the Net Protein Utilization.

levels up to 15%, the GPV can be considered constant. A criticism of the method of Heiman *et al.* is that it does not define the parameters of the base feed regime. But if one takes care to equate the levels of cellulose, phosphorus and calcium in the reference and test feeds, as

TABLE 4

*Variation in the Gross Protein Value of soya bean oil meal as a function of the feed protein level.*

| Cereal protein (%) | Test protein (%) | Feed protein level (%) | G.P.V. |
|---|---|---|---|
| 10·5 | 0 | 10·5 | 57·5 |
| 10·5 | 2 | 12·5 | 56·0 |
| 10·5 | 4 | 14·5 | 57·0 |
| 10·5 | 6 | 16·5 | 65·0 |
| 10·5 | 8 | 18·5 | 80·0 |

recommended by Carpenter, Ellinger and Shrimpton (1955) one finds good agreement between the GPV and nitrogen retention methods. This has been confirmed recently by Butterworth (1962) for cereals, by Duckworth, Woodham and McDonald (1962) for protein concentrates and by Bunyan and Woodham (1964) for fish meal.

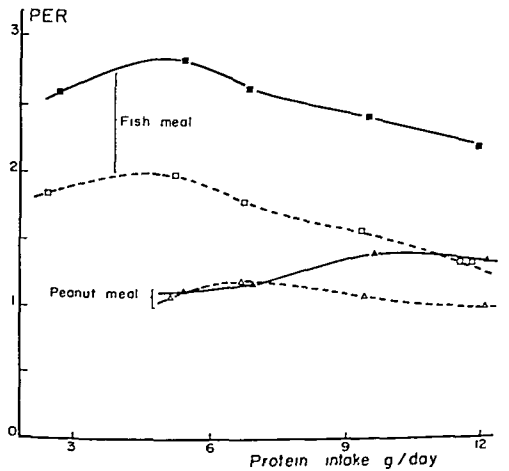


FIG. 10. Protein Efficiency Ratio (PER) as a function of amount of protein ingested under conditions of separated —— and mixed ---- *feeding.*

Study of the more sophisticated criteria for measuring protein value by means of nitrogen retention reveals the same difficulties. An excellent paper by Henry (1965) reviews the problem in the rat. The higher the feed protein level, the greater the variation in PER and the lower the NPR, while the Biological Value and NPU vary together. For protein of mediocre quality the last two criteria decrease, but for high-quality protein they remain constant as long as the feed protein level does not exceed 8%. Similar observations have been made with the chicken. Summers and Fisher (1961*a*), working with feed protein levels from 12 to 26%, observed a regular decrease in NPU. This finding, which has been confirmed by Summers, Slinger, Sibbald and Pepper (1964), holds good whatever the energy content of the feed. When the feed protein level is below 10%, the chicken behaves in a way somewhat different from the rat. De Muelenaere *et al.* (1965) reported that the NPU increases at feed protein levels up to 10% and thereafter decreases. The maximum was the same for four different proteins.

The Hohls (1955*b*) method concentrates attention on the measurement of Biological Value in the chicken at low levels of ingested nitrogen. It has the additional advantage that it permits calculation of the index proposed by Allison (1955 and 1959). Ariyoshi (1957), using adult colostomised hens, has provided supporting evidence. Tasaki and Okumura (1964) have reported that in the cock endogenous metabolic nitrogen, comprising urea, creatinine and ammonia, does not have a constant value. In particular, the excreted ammonia is more abundant on a protein-free than on a normal feed regime. Thus any method that does not rely on determination of the endogenous metabolic nitrogen has its sources of error considerably reduced. For this reason Tasaki and Okumura consider that Allison's index method is the most reliable way of estimating Biological Value with birds.

Taken together, the data show to a greater or less extent the effect of feed protein level on the various measures of protein quality. The differences in viewpoint between Summers and Fisher (1961*a*) and De Muelenaere *et al.* (1965) show that there is not yet unanimity about the choice of an optimum feed protein level for determining NPU. The same is true for PER. However, it is clear that for the majority of the methods the feed protein level must be high enough to permit growth but nevertheless below 10%. We may note, however, that Biological Value, as determined by Arnould (1961) is independent of feed protein level, at least in the rat.

2. *The feed energy level* exerts an influence on nitrogen utilization by way of the calorie/protein ratio of the feed (Donaldson, Combs & Romoser, 1956). Hohls (1958*b*) studied nitrogen retention as a function of the C/P ratio of the feed. At constant protein content, a rise in the energy content of the feed was always accompanied by a higher nitrogen gain. Similarly the maximum daily protein anabolism increased with

C/P ratio. Summers *et al.* (1964) reached the same conclusion. For each of five feed protein levels studied (from 10 to 26%), a rise in feed energy content was accompanied by improvement in NPU and in the coefficient of nitrogen retention, except at the lowest feed protein level. Ariyoshi (1957) measured digestibility and Biological Value in adult hens fed on diets of increasing energy level. As the Total Dietary Nitrogen (TDN)/protein ratio increased from 3·3 to 3·8, nitrogen digestibility did not change, but the BV rose from 50 to 65%. However, at TDN values above 66% BV did not improve. These results are in full agreement with those of Allison (1957), obtained with protein-free or complete diets. In the face of all these concordant data, the results reported by Squance and Brown (1965) are surprising; working with laying hens, they found that Biological Value and NPU improved by 20% when the energy content of the feed was reduced from 1340 to 1150 kcal/lb.

3. *The source of the non-protein nutrients* has an effect on nitrogen utilization. Many comparisons have been made of the effects of lipids with those of carbohydrates, but no substantial conclusion has been reached. Our own results (Fig. 9) indicate that there was no difference between birds receiving oil and those receiving sugar when the comparison is made between birds of the same weight. The differences in weight gain observed, if any, are the result of the effect of the nature of the ternary feed ingredient on feed intake. This is true no matter what the age of the bird (Calet, Guillaume, Delpech & Jacquot, 1964). However, there have been reports of an effect of the nature of the carbohydrate. Nitrogen utilization is improved by dextrin (Chalupa & Fisher, 1963) and by maize starch (De Muelenaere *et al.*, 1965).

4. *Feed intake* affects nitrogen utilization. It is known that feed intake is closely dependent on the energy content of the feed (Hill & Dansky, 1954) and many workers have reported variation in intake as a function of protein value (Hegsted & Haffenreffer, 1949; Sibbald, Bowland, Berg & Robblee, 1957, in the rat; Summers, Slinger, Sibbald & Pepper, 1964, in the chicken). Bender (1956) reported a correlation between feed intake and, respectively, PER and NPU. We have ourselves verified, by means of the separate feeding technique described by Calet and Melot (1961), using nine different but isonitrogenous feed regimes, that there is a high correlation between PER and feed intake. The correlation is much higher with purified proteins and proteins of vegetable origin than with fishmeal (Calet, Abraham & Baratou, 1962). This fact suggests that among protein feedstuffs, the protein in the chemical sense of the term is not the only factor affecting feed intake. Conversely, Forbes and Yohe (1955) have demonstrated beneficial effects of increasing quantities of a single ingredient on Biological Value. The interaction between the efficiency of a protein and its effect on feed intake makes the comparison of different proteins a subject full of pitfalls.

When we feed *ad libitum* a diet incorporating a protein to be tested, we must fix a certain number of experimental conditions not all of which are independent. On the one hand, the feed evokes growth in the animal, and we must take this into account when estimating nitrogen utilization efficiency. We know that the higher the growth rate, the lower the proportion of the feed used for maintenance. On the other hand, feed intake depends on the feed protein and energy contents and on the efficiency of the protein. Feed protein and energy contents must be fixed, because they determine the amounts of nitrogen and of energy ingested, and both of them influence, in turn, the efficiency of nitrogen utilization.

Two solutions to the first set of difficulties can be put forward. First, one can take account of growth rate by providing a feed regime restricted in such a way that the birds achieve the same body weight (the 'method of body weight equalization'). One then compares the amounts of feed ingested. Fevrier (1952) has argued strongly in favour of this method, notwithstanding that it is difficult to apply in practice. Secondly, one can take growth rate into account by measuring the feed requirement for maintenance and taking account of it in the calculations, as proposed by Arnould (1961).

To resolve the second set of problems, a solution has been proposed which consists of limiting the feed intake of the animals (the 'method of paired feeding'). According to Hinners and Scott (1957), the paired-feeding method yields a ranking of proteins quite different from that yielded by *ad libitum* feeding. One fixes the nitrogen intake and the energy level, but the latter limitation prevents the proteins from manifesting their full value (Moeller & Scott, 1956).

The work of Miller and Payne (1961, 1964*a*, *b*) has thrown further light on this point. When the feed nitrogen level is fixed, the nitrogen retention increases rapidly as a function of the feed energy level. But from the point at which the energy becomes surplus to requirement, the balance increases very slowly and at the price of a considerable expenditure of energy. Conversely, when the feed energy level is fixed, the nitrogen balance increases with the nitrogen supply as long as energy is not the limiting factor. From this point the nitrogen balance does not increase. These two findings show the importance of the energy/protein ratio in nitrogen utilization. Furthermore, our own work has shown that the energy/protein ratio of the feed depends not only on the quantity but also on the nature of the proteins (Calet, Jouandet & Baratou, 1961; Abraham, Calet, Rerat & Jacquot, 1961). The higher the value of the protein, the higher must the energy/protein ratio be made. Thus one can understand why restriction of feed intake hampers nitrogen utilization and why this effect is more marked with high-value than with medium-value proteins. One also appreciates why it is difficult to decide *a priori* on the energy/protein ratio of a feed that is to be used to measure protein value, because the proper ratio

is itself determined by protein value, i.e. by the very thing one is trying to measure.

In order to allow the animal to satisfy its energy needs while receiving a limited daily quantity of protein, one can put forward a method that reconciles all requirements. Sherwood and Weldon (1953), working with the rat, fed the test protein separately from the rest of the diet, which was protein-free. The dietary nitrogen supply was fixed and the nitrogen-free part of the diet is given *ad libitum*. We have used this technique with the chicken: chicks were supplied simultaneously in two separate feeders with a protein diet and a complementary nitrogen-free diet. The protein diet is given in restricted amount and the nitrogen-free diet is distributed *ad libitum* in order to equalize nitrogen intake without restricting energy intake; this procedure allows one to compare conveniently different groups on the same nitrogen intake basis (Abraham, Calet, Rerat & Jacquot, 1961). It is found that the chicken is capable of adjusting its energy intake spontaneously to achieve the appropriate energy/protein ratio. Sherwood and Weldon (1953) have shown that the animal grows more slowly under these conditions than when fed *ad libitum*. In fact, as Guillaume, Melot and Imbach (1965) have shown, the difference lies essentially in the quantity of feed consumed. When one compares the conversion coefficients of animals of the same weight fed by the two techniques, the separate-feeding method is always found to be superior. Following the same line of thought, Peretianu and Abraham (1963) have used this method with the rat. PER decreased less rapidly, as a function of feed nitrogen level, with the separate feeding system than with *ad libitum* feeding and, more important, the individual deviations were always much smaller. The separate-feeding system has been widely criticised on account of the time factor: several workers have reported that the same amount of feed separately distributed in one or several meals per day gives different growth rates. Geiger, Bancroft and Hagerty (1950) observed a reduction of nitrogen anabolism in rats during repletion when the nitrogen-free part of the diet and the protein part are allowed separately at an interval of more than 5 hours. We have recently discussed these criticisms (Calet & Albessard, 1963); they are valid when the total intake of energy is limited or when the nitrogenous feed is divided up into several nitrogen meals given at different times (Henry & Kon, 1946). However, when the energy-containing component is available *ad libitum*, the differences, if they exist, arise essentially from the amount ingested and not in any way from the value of the feed.

The separate-feeding system has other advantages. First, the nature of the growth curve is not the same with mixed as with separate feeding. The latter leads to ever-increasing weight gains as the amount of protein ingested increases, as is shown by Fig. 11. Whether the comparison be made with restricted or with *ad libitum* feeding, the growth curve of groups on the separate-feeding system is never inflected.

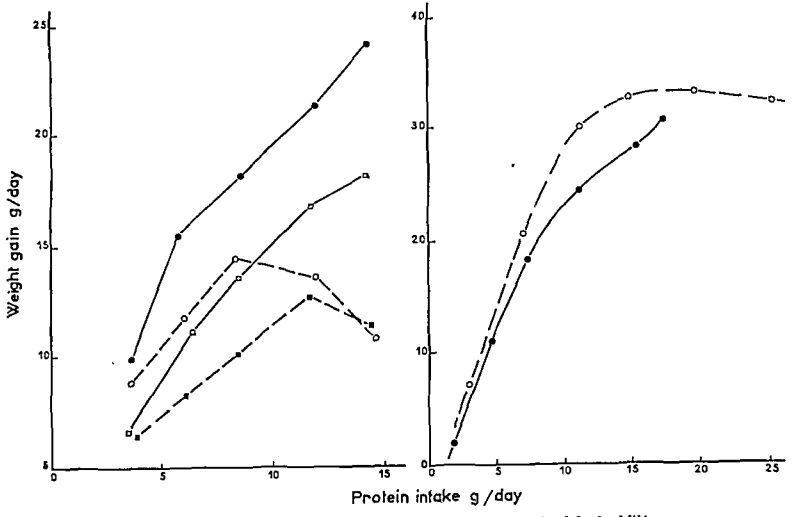


FIG. 11. Comparison of weight gains of chickens on separated ——— and — — — — mixed feeding.

Furthermore, reference to Table 2, which gives data on the body composition of birds, shows that the protein content of chickens (measured as $N \times 6{\cdot}25$) is much less variable with separate than with mixed feeding. It may be noted that in the case of peanut protein the quantity of protein ingested does not influence the body content.

Thus by not imposing on the bird an energy/protein ratio fixed in advance, and allowing it to consume the amount of energy that suits its needs, one permits the bird to form tissue protein in a way that is in harmony with the course of its growth. Thus many of the criticisms that we have levelled against NPR become invalid and one must recognize the value of this method of feeding in the estimation of protein value from weight gain. Finally, we may note that this method has been applied by Shapiro and Fisher (1965) in the determination of the nitrogen requirement of the laying hen.

## *Conclusion*

We have traced out the progressive improvements that have been introduced into estimation of protein value under the well-defined conditions of the laboratory. Abandoning the first, coarse measure, weight gain, workers have measured protein gain and the cost of body-tissue synthesis under ever more precisely defined conditions. It appears from this study that the best approximation to the measurement of protein value is given by the Biological Value, especially when one can avoid measuring the endogenous metabolic nitrogen (which is difficult to estimate), as in Hohls's method. Measurement of the slope of the regression of nitrogen retention on absorbed nitrogen, and on maximum daily protein anabolism, likewise appear to be good crtteria of protein value, notwithstanding that they are not equivalent. Within the limits of feed protein levels at which they are used, these methods allow successful comparison of feed proteins. The Net Protein Utilization method depends on the protein level of the feed. It must be treated with caution when used in conjunction with the body-water content of the bird. The body-water and nitrogen contents of the carcass depend on the growth rate and the proteins ingested. It is for this reason that methods based on weight gain appear to the theoretical worker to be imprecise.

Nevertheless, these methods are valuable to the producer, whose need is to take account of the overall efficiency of the diet of his birds. Throughout this study of the determination of the value of proteins we have scarcely considered measurement of their 'quality'. The relevant criteria have not been defined except in very circumscribed conditions. But the producer is more exacting; he needs to know not only the ability of the feed to promote growth but also its ability to yield the product that the market wants, and not only under laboratory conditions but under the conditions of his poultry house. One can

meet his needs partly by advising him to use Gross Protein Value in preference to Protein Efficiency Ratio. The former is much less variable than the latter and the conditions under which it is measured approximate more closely to those of the poultry house.

Nevertheless, this advice calls for a warning. None of the methods used is valid except at low feed protein levels and at near-zero growth rates or under conditions of feed restriction. When one departs from these conditions the measures lose their accuracy or even their sensitivity. One must therefore ask how the theoretical results can be adapted to the practical case in which, on the contrary, the birds are given diets with the highest possible protein and energy contents in order to obtain the highest possible growth rates, the lowest possible feed conversion ratios and the highest possible profits.

Thus the two different aspects of the problem of protein quality, theoretical and practical, are brought into focus, and one cannot provide a solution satisfactory from both points of view. There is no single measure of protein 'quality' which meets the needs both of the research worker and of the producer. The conditions that make a laboratory test precise are the very conditions that do not occur in the poultry house; and the conditions that occur in the poultry house are much too rough and complex to allow exact measurement. Thus one must choose one or other group of methods, depending on the object in view, knowing in advance that neither will yield complete information.

Finally, there is one aspect of feed 'quality' that has scarcely been touched upon, because we have scarcely any knowledge of it: feed aromas and their transmission to the meat and eggs. This is a field of research that has hardly been explored at all and which merits much work if the products of the poultry farmer are to satisfy the demands of the consumer.

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# 3

# OBSERVATIONS ON THE DETERMINATION OF THE 'BIOLOGICAL VALUE' OF PROTEIN SUPPLEMENTS FOR THE LAYING HEN

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*Synopsis*

Difficulties in the biological evaluation of dietary protein sources for maintenance and reproduction in the laying hen are discussed. The effect of dietary protein level on the *biological value* (BV) of a reference source of whole egg proteins is examined using colostomized laying pullets as the test animal. The fall in biological value from 95 at the 5% dietary level to 58 at the 20% level is similar to that recorded in other species.

A series of biological values is reported for mixed diets based on increasing levels of addition of a number of vegetable and animal protein supplements to a basal cereal diet. The effect of protein level on the biological values of mixed diets is also considered.

## *Introduction*

ONE of the biggest problems associated with either the determination of the biological value of a protein food or the measurement of availability of an individual amino acid in a protein food by a biological technique, is to determine the level of dietary protein which should be fed in order that comparable values for different treatment sources may be obtained from the response in terms of nitrogen balance or growth. The state of nitrogen nutrition of the test animal is bound to influence the efficiency with which nitrogen is utilized, so that the protein of a food of poorer biological value is likely to be utilized with higher efficiency than that of a food of higher biological value at the same level of protein intake. Nevertheless, there appears to be no alternative procedure other than to offer diets of similar nitrogen content.

In relation to the problem of the effect of nitrogen nutrition on the response to test protein, Miller, Carpenter, Morgan and Boyne (1965) have pointed out that, in chick growth tests of amino acid availability in which increasing levels of test proteins are incorporated, a consistent bias resulting from increased nitrogen retention with increments of test protein may not be detected. The results of work on biological value tend to suggest that such a bias may be true. As far as biological value is concerned there is ample evidence to indicate the desirability of carrying out the balance method at similar levels of protein intake for each test protein or combination of proteins. Thus Henry (1965), in a recent comparison of several methods of assessing the nutritive value of proteins, confirms the earlier observations of Mitchell (1924), Henry and Kon (1957) and of Rippon (1959) that biological value decreases with increasing protein intake and that this effect can be demonstrated for a wide variety of food protein sources. Rippon (1959) and Henry (1965) also concluded that, in general, balance methods give greater precision than the simpler growth methods and in addition provide useful data on true digestibility.

While this evidence tends to support the validity of a balance approach to the assessment of the value of food proteins for the laying hen, the conclusions of various authorities indicate that, as far as a general comparison of good-quality food protein sources was concerned, there are no consistent differences in the biological values determined using different species. Thus Mitchell (1964) states that '. . . examination of the values for different species of animals conveys the impression that specific differences are observed by other factors pertaining to experimental method, if such differences exist at all'. As pointed out by Mitchell (1964), the efficiency of protein utilization will depend on changes in the body content of amino acids during growth, and on changes in the intensity of protein synthesis with time until maturity is reached, when cellular synthesis of some amino acids may be adequate to cover the reduced requirements of the adult animal. Thus, changes in total amino acid requirement with age or with stage of reproductive process may change the dietary amino acid requirements and thus alter the biological value of a given source of protein in the diet. This was clearly shown by Henry and Kon (1957) for the sulphur amino acids in the rat and by Brooks and Thomas (1959) for lysine in the pig.

The requirement of the laying hen for amino acids is very different from that of the growing chick or rat and this would suggest that it would be necessary to establish 'biological values' for maintenance and egg production using colostomised laying hens. Such values cannot be considered comparable to those determined at low protein levels in the classical method of Mitchell (1924), since, neither the reproductive processes nor the nitrogen balance of the hen can be maintained at levels of dietary protein intake below 8 to 10% at food intakes within the capacity of the animal.

The direct application of the Thomas-Mitchell method of BV determination is not possible with laying hens because this species excretes the urine and faeces in a mixed form. In the early work of Ackerson, Blish and Mussehl (1930) on the biological value of cereal proteins, no attempt was made to separate urinary and faecal nitrogen. These authors derived an overall protein value which embraced both digestibility and biological value (BV) and allowed for a total metabolic faecal nitrogen (MFN) and endogenous urinary nitrogen (EUN) factor. This value was described by Ackerson *et al.* (1930) as a biological value on the assumption that digestibility was 100%. Van Landingham, Clark and Schneider (1942) determined in growing chicks a 'protein utilization' factor along the lines developed by Ackerson *et al.* (1930). This method also avoided the separation of urine and faeces and hence did not give separate evaluations of digestibility and biological value. Van Landingham *et al.* (1942) used the following formula for their protein utilization factor:

Protein utilization % =

$$\frac{\text{N intake} - (\text{total N excreted} - \text{body N excreted})}{\text{N intake}} \times 100$$

Although these methods give an overall value for the efficiency of utilization of a protein, they do not evaluate the relative importance of digestibility and biological value, particularly with respect to the effect of varying the protein level of the diet. For this reason, the work of MacDonald and Bose (1944) is important, as these authors attempted to use the full Thomas-Mitchell method by a chemical separation of the urinary and faecal nitrogen. Endogenous urinary nitrogen and metabolic faecal nitrogen values were obtained by chemical separation of the two components from mixed excreta of birds fed a nitrogen-free diet. Although these authors obtained results for both biological value and digestibility which correspond closely with other published data, two criticisms can be levelled at their work. The first is that chemical methods of separating urinary and faecal nitrogen are liable to much variation and the accuracy of the results obtained is entirely dependent on the similarity of the nitrogen excretion of the experimental birds to that of the birds from which the original formula was derived. A second criticism of this work is that a low level of nitrogen (750 mg/day) was fed to the hens during the experimental periods. From previous work carried out by the present authors on the determination of daily protein requirements this level of intake would only support maintenance, so that the biological values obtained may not have been related to the utilization of the dietary protein source for both maintenance and egg production (Squance & Brown, 1965).

The only investigations reported in which biological values were established by the Thomas-Mitchell technique on colostomized fowls are those of Ariyoshi (1957), Morimoto, Kubota, Ariyoshi and Hizikuro

(1961) and Squance and Brown (1965). In the work of Ariyoshi (1957), in which cocks and capons were used, the proteins of whole egg were shown to be utilized as completely as by rats, pigs and humans. In the work of Squance and Brown (1965) laying pullets were used to determine the biological value of mixed protein sources and the results showed that the amino acid deficiencies of a cereal-soya bean mixture could be demonstrated by this technique.

Following this work, the authors decided to determine the 'biological value' of commonly used protein supplements in combination with cereal proteins and to use whole egg portein as a reference material. The advisability of using a reference protein is stressed by Henry and Kon (1957) in nitrogen balance experiments of this type. It was decided to investigate the protein supplements when added to a basal cereal diet, since it is known that biological values are not additive, and it was felt that values determined on a nitrogen-free basal diet would be less useful in comparing protein supplements for practical use. In gross protein value determinations with chicks, Duckworth, Woodham and McDonald (1961) also used a cereal basal diet for the same reasons. At the same time different levels of addition of test proteins were used to study the effect of protein level on biological value. The proteins tested were white fish meal, extracted soya bean meal, extracted groundnut meal, extracted sunflower meal and meat and bone meal.

## *Materials and Methods*

In the determinations of biological values two or three colostomized laying pullets (Thornber 404) were used for each determination. The

TABLE 1

*Composition of nitrogen-free diet (Diet A) used to test reference protein*

| Ingredient | Percentage of Diet |
|---|---|
| Dextrin* | 77·91 |
| Corn oil | 12·00 |
| Mineral mix† | 5·34 |
| Vitamin mix‡ | 0·15 |
| Ground Limestone | 2·50 |
| Choline Chloride | 0·10 |
| Magnesium trisilicate | 1·00 |
| Sodium carbonate | 1·00 |

* Maize dextrin, (Harrington & Co. Ltd, London).

† Mineral mix (% of diet) $CaCO_3$, 0·3000; $Ca_3(PO_4)_2$, 2·8000; $K_2HPO_4$, 0·9000; $MgSO_4.7H_2O$, 0·2500; $Fe\,C_6H_5O_7.5H_2O$, 0·1400; $ZnCl_2$, 0·0020; KI, 0 0010; $CuSO_4\,5H_2O$, 0·0020; $H_3BO_3$, 0·0009; $CoSO_4.7H_2O$, 0·0001; $MnSO_4$, 0 0650; NaCl, 0·8800.

‡ Vitamin content IU or mg per kg diet:—vitamin A, 10,000 IU; vitamin D, 600 IU, vitamin E, 5 IU, thiamine HCl, 25; riboflavin, 16; ca pantothenate, 20; vitamin $B_{12}$, 0 02, pyridoxine HCl, 6; biotin, 0·6; folic acid, 4; inositol, 100; p-amino benzoic acid, 2, ascorbic acid, 250; niacin, 150, vitamin K activity, 12.

composition of the purified diet (Diet A) used in the study of whole egg proteins is given in Table 1 and of the cereal basal diet (Diet B) used in the study of protein supplements in Table 2. The diet C was used in

TABLE 2

*Basal diets used in the determination of biological value of individual protein supplements*

Cereal Basal Diet B

| Ingredient | Percentage of Diet |
|---|---|
| Maize meal | 50·00 |
| Ground wheat | 20·00 |
| Ground limestone | 7·00 + Maize Dextrin to 100 |
| Bone flour | 3·00 |
| Manganese sulphate | 0·03 |
| Sodium chloride | 0·50 |
| Mineral/vitamin supplement | 0·22 |

Composition of mixed diets

| Ingredient | Percentage of diet | | | |
|---|---|---|---|---|
| Supplement level (%) | 2·5 | 5 | 10 | 15 |
| Cereal basal diet | 80·75 | 80·75 | 80·75 | 80·75 |
| Maize dextrin | 16·75 | 14·25 | 9·25 | 4·25 |
| Fish meal or | 2·50 | 5·00 | 10·00 | 15·00 |
| Soya bean meal or | 2·50 | 5·00 | 10·00 | 15·00 |
| Groundnut meal or | 2·50 | 5·00 | 10·00 | 15·00 |
| Sunflower meal or | — | 5·00 | 10·00 | 15·00 |
| Meat and bone meal | — | 5·00 | 10·00 | 15·00 |

Cereal basal diet C As B with 5% soya bean meal replacing dextrin.

| Test protein source | Percentage of diet | | |
|---|---|---|---|
| Peruvian Fish Meal | 7½ | 11 | 15 |

a further study of mixed animal and vegetable protein supplements. Collection of faeces and urine was carried out by the method described by Squance and Brown (1965). True digestibilities were calculated and in the digestibility and biological value determinations metabolic faecal nitrogen (MFN) was expressed per 100 g dry matter consumed. Endogenous urinary nitrogen was calculated initially on the basis of metabolic body size, but in view of the small error involved, this procedure was replaced by a calculation based on body weight. The biological value was calculated according to the equation of Mitchell (1924).

*Results and Discussion*

The values for extracted whole egg are given in Table 3. The results of the digestibility, biological value and net protein value determinations for individual protein sources are given in Table 4. The fall

TABLE 3

*Effect of level of protein on the true digestibility (TD), biological value (BV) and net protein value (NPV) of whole egg protein added to Diet A*

(Means±S.E.M.)

| Egg Protein per cent. | TD | BV | NPV |
|---|---|---|---|
| 5 | 93·0±0·15 | 94·8±2·80 | 88·2 |
| 10 | 88·6±1·60 | 78·0±2·41 | 69·3 |
| 15 | 90·5±0·22 | 61·0±4·14 | 55·2 |
| 20 | 88·0±1·26 | 57·8±2·37 | 50·8 |
| Mean | 89·9 | 71·5 | 64·4 |

TABLE 4

*Effect of level of protein on the true digestibility (TD), biological value (BV) and net protein value (NPV) of fishmeal, soya bean meal, groundnut meal, sunflower meal, meat and bone meal in combination with Diet B*

(Means±S.E.M.)

| Supplement added per cent. | | TD | BV | NPV |
|---|---|---|---|---|
| White fish meal | 2·5 | 92·8± | 59·2± | 55·0 |
| | 5·0 | 91·6±0·6 | 64·9±1·7 | 59·5 |
| | 10·0 | 90·7±1·3 | 62·9±4·0 | 56·9 |
| | 15·0 | 91·2 | 65·4 | 59·7 |
| | Mean | 91·6 | 63·1 | 57·9 |
| Soya bean meal | 2·5 | 91·2±1·0 | 65·3±2·3 | 59·6 |
| | 5·0 | 86·0±1·4 | 56·5±6·0 | 48·5 |
| | 10·0 | 91·7±0·4 | 62·5±3·5 | 57·4 |
| | 15·0 | 91·0±0·1 | 66·4±2·7 | 60·5 |
| | Mean | 89·9 | 62·5 | 56·2 |
| Groundnut meal | 2·5 | 91·4±1·0 | 60·9±2·8 | 55·7 |
| | 5·0 | 92·2±1·1 | 60·1±3·3 | 55·5 |
| | 10·0 | 92·0±0·5 | 56·2±1·0 | 51·8 |
| | 15·0 | 91·8 | 55·5 | 50·9 |
| | Mean | 91·9 | 58·6 | 58·6 |
| *Sunflower meal | 5·0 | 89·6 | 65·1 | 60·5 |
| | 10·0 | 91·1±2·3 | 53·5±0·3 | 48·7±1·4 |
| | 15·0 | 93·0 | 63·5 | 59·1 |
| | Mean | 91·0 | 58 8 | 54·2 |
| *Meat and bone meal | 5·0 | 87·9±1·5 | 47·6±4·0 | 41·5±3·2 |
| | 10·0 | 87·0±0·9 | 49·1±2·6 | 44·4±3 1 |
| | 15·0 | 83·5±2·8 | 51·4±1·8 | 43·1±2 8 |
| | Mean | 85·9 | 49·2 | 42 9 |

* In the calculation of BV, endogenous urinary nitrogen (EUN) was expressed as mg N/kg body weight and not in terms of metabolic body size.

vegetable proteins. There is also the possibility that body reserves of protein may compensate for deficiencies at the lower levels of intake by the laying pullet though the net nitrogen balance in almost all cases reported here was positive.

With a view to examining the effects of protein level on mixed protein diets biological value tests of diets of different protein contents were determined and the results which we have obtained so far for such diets are given in Table 5. The highest level of protein tested namely 17·6% gave the lowest value for biological value in this series of tests but there is no general indication that protein level influences BV in the case of these practical diets.

TABLE 5

*True digestibility (TD), biological value (BV) and net protein values of mixed cereal-protein diets of different protein content*

| Protein source | Per cent. | No. of diets tested | BV | NPV |
|---|---|---|---|---|
| Cereal, white fish meal | 13·2 | 2 | 62·3 | 55·6 |
| | 15·1 | 1 | 55·0 | 50·6 |
| | 17·0 | 3 | 58·2 | 53·3 |
| Cereal, soya bean meal | 14·2 | 4 | 59·3 | 54·4 |
| Cereal-Soya bean diet C, | 11·8 | 1 | 64·9 | 59·2 |
| with different levels of | 16·0 | 1 | 65·5 | 58·4 |
| Peruvian fish meal | 17·6 | 1 | 48·4 | 40·7 |

In the tests of individual proteins added at the 2·5 and 5·0% levels to the cereal base the diets were deficient in at least five essential amino acids on the basis of the standards recommended by A.R.C. (1963). At the 10% level of addition all the supplements were deficient in lysine, methionine and isoleucine, but at the 15% level, fish meal was adequate in all amino acids and soya bean was deficient only in methionine. At this level the two highest biological values obtained in the present work were with this level of addition of white fish meal and soya bean meal.

In general, indications from this work are that biological values determined with the colostomized fowl are capable of ranking individual sources of proteins fairly satisfactorily while at the same time giving information on true digestibility. One of the difficulties in pursuing this type of work with a view to establishing mean biological values for different proteins resides in the technical problems associated with balance work using colostomized fowls. In future work the authors feel that this method could provide reliable information on the biological value of individual proteins determined without the complicating influence of cereal proteins. The extension of the pre-experimental and balance periods to eight days could be recommended on the basis that this would reduce the errors in the determination of biological value. Indications from the present work are that it is essential to

in biological value, with increased protein intake demonstrated by Henry and Kon (1957) in the rat, and by Ackerson *et al.* (1930) in the fowl is confirmed. The maximum value of 95% for the biological value of whole egg protein is similar to that obtained by Henry and Kon (1957) and Ariyoshi (1957). The laying hen, therefore, appears to metabolise whole egg protein in a similar manner to other species. The trend in the biological values associated with increased dietary egg protein is similar to that recorded by Henry (1965).

In agreement with previous work by Squance and Brown (1965) the results showed that true digestibility was similar at all levels of protein addition for the various supplements. In the case of meat and bone meal a lower mean digestibility of 86% was observed and this was thought to be associated with the high level of ash present in these diets.

The data for the utilization of proteins added to the basal cereal diet show no consistent trends except that, in all cases apart from meat and bone meal, the biological values of the supplemented diets at all levels were significantly different from that of the cereal basal diet. The mean values obtained for the various cereal-protein mixtures showed that the figure of 49·2 for the biological value of meat and bone meal was the only low value obtained, all the other figures lying between 55 and 67. Previous work by Squance and Brown (1965) had shown that practical diets capable of supporting optimum egg production gave a mean biological value of 58·9 and this figure could be considered as typical of practical diets used for the laying hen. There is support for this statement from the work of Morimoto *et al.* (1961) who quote a figure of 60% for Japanese commercial diets.

There was a high error in the estimate of 47 for the biological value of the basal cereal diet due to the abnormal urinary nitrogen figure obtained in one of the test animals. The effect of increasing dietary protein level on the biological value for laying hens is not nearly so marked as in the case of the studies reported in the rat on diets varying from 4 to 16% protein (Henry & Kon, 1957). This smaller effect of protein level on the biological value could be explained by the fact that, in a constant mixed cereal source with increasing levels of supplement, the relative amino acid balance will be changing with incremental additions of the test protein. In some instances, notably in the case of the soya bean and sunflower proteins, there is an indication that the second level of addition of the protein tended to depress the biological value. In the case of the animal proteins this effect was not apparent. Henry and Kon (1957) have commented on the fact, in classical biological value tests with rats, a deficiency of lysine, which is essential for growth, may not be so detrimental to the utilization of nitrogen in the test group on the lowest level of protein because of their lower rate of growth. It could well be that depression of egg protein formation at the lower protein levels is revealed in a similar manner in the case of the

vegetable proteins. There is also the possibility that body reserves of protein may compensate for deficiencies at the lower levels of intake by the laying pullet though the net nitrogen balance in almost all cases reported here was positive.

With a view to examining the effects of protein level on mixed protein diets biological value tests of diets of different protein contents were determined and the results which we have obtained so far for such diets are given in Table 5. The highest level of protein tested namely 17·6% gave the lowest value for biological value in this series of tests but there is no general indication that protein level influences BV in the case of these practical diets.

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| Cereal, soya bean meal | 14·2 | 4 | 59·3 | 54·4 |
| Cereal-Soya bean diet C, with different levels of Peruvian fish meal | 11·8 | 1 | 64·9 | 59·2 |
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| | 17·6 | 1 | 48·4 | 40·7 |

In the tests of individual proteins added at the 2·5 and 5·0% levels to the cereal base the diets were deficient in at least five essential amino acids on the basis of the standards recommended by A.R.C. (1963). At the 10% level of addition all the supplements were deficient in lysine, methionine and isoleucine, but at the 15% level, fish meal was adequate in all amino acids and soya bean was deficient only in methionine. At this level the two highest biological values obtained in the present work were with this level of addition of white fish meal and soya bean meal.

In general, indications from this work are that biological values determined with the colostomized fowl are capable of ranking individual sources of proteins fairly satisfactorily while at the same time giving information on true digestibility. One of the difficulties in pursuing this type of work with a view to establishing mean biological values for different proteins resides in the technical problems associated with balance work using colostomized fowls. In future work the authors feel that this method could provide reliable information on the biological value of individual proteins determined without the complicating influence of cereal proteins. The extension of the pre-experimental and balance periods to eight days could be recommended on the basis that this would reduce the errors in the determination of biological value. Indications from the present work are that it is essential to

provide at least 13 g of protein per day in order to maintain positive balance at the levels of egg production normally encountered in this work.

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# 4

# PLASMA AMINO ACID LEVELS

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*Synopsis*

It is suggested that there is little value in contemplating directly using plasma amino acid levels either as an index of the quality of dietary protein or of the quantitative needs of the animal. It is more profitable to use relative amino acid patterns to identify an ideal amino acid balance through the ability to recognise the metabolic consequences of imbalance.

Attempts to measure the nutritive value of proteins have not yet led to a generally accepted procedure. The concept of ascribing to a protein a value is inherently non-specific for though in the case of vitamins or minerals a single essential nutrient is considered at any one time, in any assay of protein value a multitude of nutrients must be simultaneously evaluated. Most conventional procedures of protein assessment are rather empirical and are based upon growth, efficiency of food utilization or nitrogen balance as an index of the adequacy of dietary protein. However, in those assay methods based upon protein regeneration an attempt is made to take a more functional outlook. There is still a very critical need for a sensitive procedure to establish the relative adequacy or otherwise of a dietary protein to meet the needs of the animal. The determination of plasma amino acid levels might well be developed to constitute such an index.

The use of automatic equipment for the assay of amino acids by ion-exchange chromatography has made it possible on a routine basis to determine blood amino acid levels. Plasma amino acid patterns are affected by many variables most of which are not clearly understood. There is of course a regulation by the intake of amino acids, composition of dietary protein, pattern of amino acid release during digestion, rate of absorption—and also a modification by the extent to which amino acids are metabolised by intestinal tissues during absorption. This latter factor and the effect of the mixture present upon the absorption process may account for the frequent lack of any obvious correlation between the concentration of amino acids in the portal plasma and the quantities ingested (Peraino & Harper, 1963). In addition to the rate of entry into plasma, the removal of amino acids from plasma also

regulates the level—removal by entry into cellular matter and by passage along both anabolic and catabolic routes. Since the actual amino acid level at any one time—as with all intermediates in a dynamic system—merely represents a small balance between major entries and withdrawals it is difficult to accord to a single value an absolute meaning. Yet it may be appropriate to draw conclusions from relative changes.

Since the amino acid composition of the dietary protein is only one of many factors affecting plasma amino acid levels it would hardly seem possible to postulate a direct or simple relationship between the two. Some limited success was apparently obtained by Jarowski (1961) in calculating daily requirements for amino acids using a formula involving the actual amino acid level. In the same way McLaughlan (1963) demonstrated a relationship between protein quality and plasma amino acid levels. From a theoretical consideration there would, however, seem to be little hope of directly using plasma amino acid levels either as an index of the quality of protein fed or of the quantitative amino acid needs of the animal.

It would seem to be a more profitable proposition to contemplate the use of relative amino acid patterns to identify an ideal amino acid balance through the ability to recognize the metabolic consequence of any imbalance. By the identification of a satisfactory balance it might be possible to establish allowances for best chick growth, and by following the pattern of change of plasma amino acid levels resulting from the intake of a diet recognized to be imbalanced in terms of amino acid supply, information might be gained on the mechanism that brings about the known consequences of imbalanced diets.

It is reasonable to suppose that when there is a surplus of a particular amino acid in the diet its level within blood plasma tends to increase. As a corollary, the thesis can be tested that when there is an ideal dietary amino acid balance and when the total protein intake is held at a relatively low level, one might expect individual plasma amino acid concentrations and the total plasma amino-nitrogen concentration to be minimal.

The subject of amino acid imbalance has recently been reviewed (Harper, 1964; Harper, 1965; Lewis, 1965). The viewpoint was put forward by Lewis (1965) that the overall phenomenon of amino acid imbalance could be regarded as the result of an interaction between pairs or groups of amino acids. The amino acid which must be added to counteract the growth depression resulting from the intake of the imbalanced diet might be regarded as the target of an unknown mechanism which results in the requirement for that amino acid being increased. The agent of the interaction can be defined as the amino acid addition of which leads to the phenomenon. The determination of plasma amino acid levels under these conditions could well throw light upon the mechanisms bringing about the phenomena.

Lewis (1965) also discussed the importance of catabolic processes in

this context—that the agent amino acid present in the diet in excess might encourage an increase in oxidative catabolism which carries with it the limiting or target amino acid. In this way one would expect a substantial fall in the concentration in blood plasma of the target amino acid, and also to be able to identify any potential alternative targets. No major effect would be expected upon most of the other essential amino acids. On the other hand Harper (1965) suggested a mechanism that might be regarded as an anabolic imbalance, wherein the added amino acid by resulting in an increased plasma concentration, stimulated protein synthesis in the first instance and so led to

TABLE I

*Effect of amino acid deficiencies on free amino acids in blood plasma (mg/100 ml). Results obtained from Hill and Olsen (1963)*

| | Leucine | Valine | Arginine | Threonine | Lysine |
|---|---|---|---|---|---|
| Basal diet (9·5% protein) | 2·3 | 1·9 | 4·3 | 1·0 | 2·8 |
| +amino acids without leucine | 0·6 | 13·6 | 6·2 | 15·1 | 11·0 |
| +amino acids without valine | 2·8 | 1·0 | 3·8 | 15·7 | 9·2 |
| +amino acids without arginine | 3·6 | 7·4 | 1·2 | 25·5 | 10·9 |
| +amino acids (complete) | 3·3 | 6·6 | 8·7 | 10·6 | 7·9 |

an aggravation of the relative inadequacy of the most limiting amino acid for other functions. Were this the case an elevated plasma level would be expected in the case of the so-called agent and a substantial drop in the case of most other amino acids. These concepts can be examined in the light of some of the data now available upon plasma amino acid levels under different conditions.

There is considerable evidence now available to show that the identity of the first limiting amino acid can probably be established by plasma amino acid determination. Thus Hill and Olsen (1963) added various amino acid mixtures to a low protein diet (based upon isolated soya bean protein). They compared plasma amino acid levels when a complete mixture was fed and when particular amino acids were omitted. Some of the results are given in Table 1: it seems that merely seeking minimal individual plasma amino acid levels is not adequate to obtain an ideal dietary amino acid balance. However, overall plasma amino acid levels or total amino nitrogen might constitute a better index: thus Askelson and Balloun (1963) concluded that chicks fed a methionine supplemented isolated soya bean protein diet demonstrated increased weight gains and, in general, lower plasma concentrations of free amino acids than did chicks fed the diet not so supplemented. Conversely Gray, Olsen, Hill and Branion (1960) showed that when a lysine-deficient diet was fed the plasma lysine level was reduced whereas in the case of most other amino acids the plasma

level was greater (Table 2). It is difficult to account for the fall in arginine.

Some light is thrown upon the mechanism of the lysine-arginine interaction in the chick by the work of Jones (1964). Some of his

TABLE 2

*Effect of feeding lysine-deficient diet on blood plasma amino acids (mg/100 ml). Results from Gray et al. (1960), mean of two experiments*

| | Lysine adequate | Lysine deficient |
|---|---|---|
| Lysine | 4·4 | 1·1 |
| Threonine | 6·4 | 16·0 |
| Tyrosine | 2·5 | 4·0 |
| Arginine | 9·4 | 6·0 |
| Glycine | 5·0 | 5·3 |
| Histidine | 3·0 | 3·1 |
| Valine | 5·6 | 5·4 |
| Isoleucine | 2·5 | 2·2 |
| Phenylalanine | 2·5 | 3·0 |
| Total (excluding lysine) | 36·9 | 45·0 |

results (Table 3) show that when excess lysine is fed the plasma lysine level increases greatly, the arginine level falls and all other plasma amino acid levels are slightly raised. This suggests that arginine is the

TABLE 3

*Plasma amino acids (μ moles/100 ml) when a diet based upon soya bean protein is fed and when a lysine excess is added. Results from Jones (1964).*

| | Standard diet | Plus 2% L-lysine |
|---|---|---|
| Aspartic acid | 2 | 5 |
| Threonine | 22 | 30 |
| Serine | 83 | 96 |
| Glutamic acid | 15 | 24 |
| Glycine | 37 | 51 |
| Alanine | 44 | 64 |
| Valine | 25 | 33 |
| Methionine | 6 | 9 |
| Isoleucine | 9 | 19 |
| Leucine | 18 | 26 |
| Tyrosine | 17 | 21 |
| Phenylalanine | 11 | 15 |
| Lysine | 39 | 268 |
| Histidine | 10 | 13 |
| Arginine | 22 | 17 |

only target and that the mechanism can be considered to be one of catabolic imbalance.

It is possible to prepare a chick diet based upon maize and sesame meal that is specifically deficient in lysine. In a recent experiment

carried out at the University of Nottingham by Mr D. Hewitt, graded supplements of lysine were added to such a basal diet up to a likely level of adequacy and also further to a point of slight surplus: the lysine content ranged from 0·7 to 1·4% of the diet. A selection of the results, growth performance and plasma amino acids, is given in Table 4.

TABLE 4

*Growth rate (g/day) and plasma amino acid levels (μ moles/100 ml) on graded supplementation of a maize-sesame meal diet with L-lysine (Hewitt, unpublished)*

| Dietary lysine | Growth (g/day) | Lysine | Arginine | Histidine | Isoleucine | Leucine | Phenyl-alanine |
|---|---|---|---|---|---|---|---|
| 0·7 | 15·1 | 7·0 | 25·5 | 28·5 | 19·5 | 23·5 | 26·8 |
| 0·8 | 18·8 | 13·5 | 29·2 | 21·0 | 16·8 | 24·3 | 25·1 |
| 0·9 | 20·9 | 22·5 | 26·3 | 23·3 | 16·8 | 21·8 | 21·3 |
| 1·0 | 22·5 | 28·5 | 25·5 | 18·5 | 11·0 | 21·3 | 19·4 |
| 1·2 | 21·8 | 35·5 | 22·3 | 18·5 | 18·8 | 23·5 | 21·5 |
| 1·4 | 19·5 | 84·2 | 14·8 | 21·3 | 19·6 | 22·8 | 28·4 |

There is a steady increase in the plasma lysine approximately in parallel with dietary lysine. It is of interest to note that overall plasma levels seem to fall up to the point at which the lysine intake is equivalent to 1% of the diet and at higher levels rise again. This trough in the plasma level of amino acids other than lysine may indicate the most satisfactory level of dietary inclusion of lysine. The drop in plasma arginine at the point of highest dietary lysine confirms the involvement of lysine and arginine in a catabolic pattern of interaction.

Though the studies of Jones (1964) and of Smith and Lewis (1966) suggest there is a specific interaction between lysine and arginine and give no indication of an alternative target to arginine Winje, Harper, Benton, Boldt and Elvejhem (1954) demonstrated the existence of a lysine-histidine interrelationship and Rosenberg, Culik and Eckert (1959) of a lysine-threonine interaction. Mr. J. P. F. D'Mello at Nottingham University has examined these two alternative interactions in chick trials involving a recording of growth performance and the determination of plasma amino acid levels. He prepared two basal diets; in the first threonine was arranged to be the first limiting amino acid with arginine as the second limiting whilst in the second the intended sequence of limitation was histidine followed by arginine. The objectives were to throw light on the general circumstance of amino acid interactions, to demonstrate whether there were any alternative targets to arginine when lysine was the agent and to establish whether the lysine-arginine interaction could be demonstrated even when arginine was not the first limiting amino acid.

Some of the results of these two trials are presented in Tables 5 and 6. In the first experiment (Table 5) it was demonstrated that threonine was the first limiting amino acid, that lysine supplementation gave a

growth depression which was only clearly reversed by adding arginine. The plasma amino acid data show there is only a clear depression in arginine on supplementation with lysine and confirm the specific involvement of lysine and arginine in a catabolic interaction. In the

TABLE 5

*Growth rate (g/day) and plasma amino acid levels (μ moles/100 ml) on amino acid supplementation of a basal diet, threonine first limiting and arginine second (D'Mello, unpublished)*

| Amino acid supplement | Growth (g/day) | Lysine | Arginine | Histidine | Threonine | Iso-leucine | Leucine | Phenyl-alanine |
|---|---|---|---|---|---|---|---|---|
| Basal | 12·9 | 83·8 | 16·6 | 22·8 | 26·6 | 15·8 | 22·4 | 12·4 |
| +Arginine | 12·9 | 91·4 | 20·0 | 17·4 | 23·4 | 12·0 | 22·0 | 12·0 |
| +Threonine | 19·5 | 84·8 | 18·5 | 21·8 | 88·4 | 14·8 | 20·6 | 12·9 |
| +Arg+Thr | 20·6 | 80·0 | 28·8 | 12·5 | 94·8 | 15·4 | 23·6 | 12·0 |
| +Lysine | 7·8 | 119·8 | 7·4 | 19·6 | 33·6 | 11·8 | 19·0 | 10·0 |
| +Lys+Arg | 12·8 | 169·6 | 10·4 | 16·9 | 40·4 | 12·8 | 23·8 | 11·8 |
| +Lys+Thr | 9·8 | 115·0 | 6·8 | 11·5 | 133·6 | 10·8 | 18·8 | 10·2 |
| +Lys+Arg+Thr | 16·7 | 120·0 | 7·6 | 12·0 | 80·0 | 9·0 | 18·2 | 8·6 |

second experiment (Table 6) the identity of histidine as first limiting amino acid is not too readily established but the growth inhibition by lysine and its correction by arginine is again apparent. On adding lysine there is again a clear depression in the plasma arginine level and an increase in the concentration of most other amino acids. When

TABLE 6

*Growth rate (g/day) and plasma amino acid levels (μ moles/100 ml) on amino acid supplementation of a basal diet, histidine first limiting and arginine second (D'Mello, unpublished)*

| Amino acid supplement | Growth (g/day) | Lysine | Arginine | Histidine | Iso-leucine | Leucine | Phenyl-alanine |
|---|---|---|---|---|---|---|---|
| Basal | 19·5 | 62·4 | 14·6 | 19·2 | 17·6 | 21·4 | 13·8 |
| +Arginine | 19·8 | 56·8 | 26·8 | 18·8 | 16·4 | 19·5 | 12·8 |
| +Arg+His | 20·9 | 55·4 | 30·0 | 25·8 | 14·2 | 19·4 | 11·8 |
| +Lys | 13·9 | 146·2 | 7·4 | 21·4 | 18·8 | 22·7 | 14·8 |
| +Lys+Arg | 17·8 | 127·0 | 17·0 | 19·0 | 15·8 | 19·4 | 13·4 |
| +Lys+His | 9·5 | 133·0 | 8·4 | 27·8 | 20·2 | 24·0 | 12·4 |

arginine was also included the overall plasma levels dropped, presumably again reflecting a better dietary amino acid balance.

It seems clear that the use of plasma amino acid determinations can lead to a more sensitive index of dietary protein suitability and that it can encourage a more functional rather than an empirical outlook in describing the consequences of a lack of ideal dietary amino acid balance.

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# DISCUSSION ON PART I

*Mr W. S. Miller of Reading:* The emphasis this morning has been on the assessment of the nutritive value of proteins for poultry, and I think the papers have brought out quite clearly how it is possible to assess nutritive value in a number of different ways, using two main approaches. The first is an analytical approach, which attempts to assess for individual acids the amounts present and their availability to, and utilisation by, the animal. The other approach attempts to assess the nutritive value of a protein as an overall evaluation based on feeding tests with animals. It will be clear from the excellent papers we have had this morning that these two approaches are complementary and that the improved methods of amino acid estimation that Dr E. L. Miller explained have not superseded biological tests, although the emphasis may have to be on slightly different aspects than has hitherto been the case. It is certain that no one approach or single method of evaluation can provide the answers required for all circumstances. The approach which is made to protein evaluation and the method chosen will depend very much on what purpose is in mind. In fact the application of these methods to particular problems is something which the speakers did not go into very fully, but in a sense this is the crux of the matter. It seems to me that there are three main types of application for which suitable means of assessing the nutritive value of proteins are required. In the first place we have the problem of selecting a particular feed (or feeds) from those available, so as to make up a diet which in any particular circumstance will be the most economical. Secondly, having decided on a particular food to supply the protein of the diet in given circumstances, we would need to be able to assess the difference between good and bad samples of a particular kind of feeding stuff. And how are we going to define what is good and what is bad in a particular sample? In the third place, we need basic information on how to formulate from available materials the diet to meet the needs of a particular class of stock.

It would seem that the more logical approach would be the analytical one, i.e. determination of the individual amino acids in the food. From the analytical results, together with information about amino acid requirements, the nutritive value in different circumstances of different feeds and combinations of feeds could be estimated. This procedure is, of course, fraught with many errors and difficulties, and these have been brought out. No doubt we shall hear later in this conference about possible shortcomings in our estimates of actual amino acid requirements of poultry.

In his paper, Dr Miller showed very clearly how the assessment of gross amino acid composition by ion exchange chromatography, or

other methods involving the complete hydrolysis of the protein, does not necessarily indicate that a sample of a feedingstuff is good or bad. We have to seek a method to measure available amino acids. He was very careful, I thought, in defining what he meant by availability, and I wonder (although the definition was excellent in the context of his paper), whether in fact it was too restrictive. He pointed out that only for lysine is there at present a chemical test for availability. To assess availability of other amino acids it is necessary to use microbiological methods, and the validity of chemical or microbiological tests will depend finally on biological tests.

The chief drawback, however, to bioassays based on growth is their very low precision. In our experience at Reading the most we could ever hope to achieve would be values for certain amino acids within limits of plus or minus 20%. But in practical trials, the likely limits would be plus or minus 30% and, on some occasions, plus or minus 50%. Hence the results of such tests have very limited meaning and one might ask whether we are going about the task in the right way. Should we use bioassays to assess whether or not the results from microbiological and chemical tests are valid, or should we use biological assays to demonstrate possible differences between particular proteins in a given dietary situation, where one specific amino acid is limiting? When attempts are made to compare such responses with the responses of animals given the pure amino acid, the error, or increase in error, of this measurement is very considerable.

Another issue is whether parameters alternative to weight gain or gain/feed ratio would result in greater precision. The problem of what parameter to use in assessing feeding tests has been raised by the other three main speakers this morning. Dr Calet emphasized that very many factors can affect protein utilization and that all these various factors have to be isolated in assessing nutritive value. As he said, the limitations of conventional tests are that they must be done under very standardized conditions. I thought his results on body composition, rate of growth and level of protein were particularly interesting, and revealed how careful we must be in interpreting assays based on weight gain of chickens or on nitrogen content of carcasses based on water determinations.

I was particularly grateful to him for introducing the work of Arnould, with which I was not familiar, although I didn't grasp all its significance. In the concepts of Arnould we have a means to resolve the several variables which can influence protein utilization and to make some progress in assessing nutritive value. Choice of parameter was also brought up by Professor Brown, in assessing the nutritive value of proteins for laying hens; of course, weight gain cannot be used. Here, a biological value determination is very interesting as there are so very few data involving classical methods applied to poultry. He asked whether it was worth going on with this work. I suggest that it is very

worth while to do so, but not necessarily on the lines of earlier work i.e. trying to characterize feeds with biological value, but rather to use this parameter as a means of establishing the relevance of various chemical, microbiological and other tests for measuring protein quality. I think that the reason why he didn't obtain a decline in biological value when he increased the protein level in his practical diets is that he was increasing the proportion of the protein concentrate to that of the cereal. With the different levels of protein he wasn't in fact getting the biological value of the same dietary protein mixture. This just brings out again another of the very complicating factors in practical protein nutrition, in that it is so very difficult in these studies to alter only one variable at a time.

Finally, we had a most interesting paper from Professor Lewis, where he considered some possibilities of using plasma amino acid levels as a nutritional parameter. Here again, such levels are subject to a number of almost opposing factors and I gathered that he was somewhat despondent about using these levels to assess the nutritive value of dietary protein as such, or as a parameter for availability of amino acids. But I wonder whether, instead of chickens being fed *ad libitum* in this type of work, very standardized and rigid feeding conditions would result in more meaningful blood determinations on chickens. It seems to me that the time interval between the last meal and withdrawal of blood will be a very complicating factor in interpretation. The results are interesting and show how this work might develop in the future.

*Professor D. C. Snetsinger (Minnesota):* I would like Professor Lewis to say a little more about the technique he used for sampling the plasma. Did he take samples immediately after feeding *ad libitum* or was there a fixed time interval? Could the technique be improved by giving a specified amount of food and drawing the blood sample after two or three hours, whichever would be desirable?

*Professor D. Lewis (Nottingham):* A standardized procedure was used in these experiments. The general principle was that birds were put on the experimental diets at one week of age, for the next two weeks. The samples were drawn towards the end of the acclimatization period. An effort was made to equalize the situation in relation to period of feeding by withholding food until the birds were reasonably hungry; they were then allowed to have food for another defined period and the sample was taken a specific time after that. We thought that further variability could perhaps be minimized by replication. We tried to make the tests as comparable as possible in terms of the period of acclimatization to the diet and the time interval between feeding and withdrawing the blood.

*Mr T. R. Morris (Reading):* Could I ask a supplementary question? Was it peripheral blood or portal blood that was sampled in this case?

*Professor D. Lewis:* Peripheral blood.

*Mr Morris:* May I ask Dr Brown to define more precisely his use of the term biological? In what way was the reference diet brought in?

*Professsor W. O. Brown (Belfast):* The data from the reference diet are not brought in at all. Biological value, in my usage, follows the classical Thomas-Mitchell definition of percentage of retained nitrogen divided by the nitrogen.

*Mr Morris:* Surely, if the protein level is raised, there must come a point at which the percentage retention will go down and the biological value will decline. Equally, when the protein in the diet is raised beyond the requirements of the animal, the biological value must decline—it can't do anything else.

*A speaker:* Mr. Chairman, that is perhaps an oversimplification, because there are two ways in which this type of phenomenon could occur. It has been shown conclusively by Henry M. Cohen (?) that at the lower levels of protein intake different responses will be given by an aged rat and a young rat. Of course if an animal is growing very slowly the demand for individual amino acids will be lower; whether the slow growth is a reflection of a deficiency or an aged rat doesn't apparently matter. In this situation, again, 'demand' can affect 'need'. Is it not obvious, that there must be a ceiling to the phenomenon although at 20% dietary protein it is not of much interest practically?

*Mr W. R. Muir (Glaxo Laboratories):* Dr Miller said that there was a good correlation between the chemical tests for available lysine and protein nutritive value as measured by the microbiological assay. It is true that, over a given group of samples, good correlation can be seen, but samples can also be obtained where good correlation does not occur. This happens particularly where there is a loss of cystine during the processing, so the ratio of cystine to lysine falls. In my experience this loss of cystine occurs to a variable extent in the manufacture of fishmeal and also during the removal of solvent from oil seed proteins.

*Dr E. L. Miller (Cambridge):* My only comment is that the correlation which we have recorded was specifically for meat and fishmeals of the types we have studied so far, and in these particular materials the cystine content is usually fairly low.

*Dr N. A. Matheson (Rowett Research Institute, Aberdeen):* I wonder if the speakers and Dr Miller in particular would comment on the question of available amino acids. Some reports state that even amino acids like leucine are unavailable, but it is difficult to imagine any chemical reaction which would make leucine unavailable. Is the concept of unavailability due to chemical reaction more widely applicable to lysine or is the idea in fact based on a misconception?

*Dr E. L. Miller:* Under certain mild processing conditions, when carbonyl groups are formed from carbohydrates or possibly from breakdown products of oxidized fat, the lysine will bind and become

unavailable without affecting the availability of other amino acids. We have particularly studied methionine in this respect and have clearly shown something like a 25% drop in available lysine on mild heating of glucose mixtures without any change in the methionine. Using the microbiological assay procedure for other amino acids, we confirmed that no other amino acids were affected. On the other hand, much more severe heat treatments, either in the absence or in the presence of carbonyl groups, results in reduced availability of methionine and in some instances from 3·4 down to 0·4. There is evidence that other amino acids may be similarly affected. Thus bioassays for isoleucine on heated material in which heat treatment produced a 30% drop in availability of methionine also produced a drop of 20% in the availability of isoleucine. It appeared when we tested these materials on the rat that there was only a much smaller drop in nitrogen digestibility. With the colostomized chick, in which the faeces could readily be collected, only a small drop in the digestibility of the nitrogen, was observed, too little to account for the changes in availability. In our view the lysine and possibly other amino acids with reactive groups, for instance arginine, may form resistant linkages which inhibit enzymic attack, and therefore other amino acids which happen to be adjacent to lysine or held within a peptide will suffer in their availability.

*Mr C. J. L. Baker* (*Ministry of Agriculture, Fisheries and Food, Cambridge*): I have a very simple question which perhaps Professor Brown would like to answer. Ever since I can remember, which is some time now, experimental animals are fed and just weighed. This has been open to some criticism in view of the fact that different tissues vary in composition. I wonder whether, in the case of a chick, simple weighing is even less justifiable, since there is the complication of feather growth? Would it be possible that, in circumstances where one amino acid is limiting, the demand for feather growth might first be met, to the detriment of body growth?

*Professor Brown:* We felt that in our factorial estimate of protein requirements for the hen, feather growth was a factor that should be taken into account, but we have not as yet been able to find any useful information. In fact, for the modern laying hen it appears that the feathers do not change much in total weight and feather growth makes quite small demands. Nevertheless, during growth and reproduction there must be a gain in feather weight and this, plus the actual gain in body weight, must count in assessing nitrogen requirements. How far this might affect the need for an individual amino acid, is not known.

Returning to the question of amino acid availability, the classical definition relates to absorption from the alimentary tract. Dr Miller's definition on the other hand relates to the potency of the protein source to support growth under conditions in which only one amino acid was limiting. This is a different and much more complicated

concept than simple availability of a nutrient in the tract, since it also involves utilization of the amino acid by body tissue.

*Dr A. H. Sykes (Wye):* I was very interested in the data provided by Professor Lewis on the relationship between lysine and arginine, and I wondered whether there was any relationship between the amino acid imbalance in the various experiments he reported and the possible function of arginine in kidney tissue. Could it be involved in the detoxication mechanism through ornithine?

*Professor D. Lewis:* That possibility has exercised the minds of many concerned with the requirements for arginine. The available information is that the suggested pathway is inevitable and that any demands it makes can be met. If we consider the production of ornithine in order to detoxicate benzoic acid and produce ornithuric acid, there is anyhow an excess of arginase. A wasteful route involving a surplus would scarcely cause imbalance.

May I add a little to the discussion on feathers. The undesirable products of a chick are to be accepted; one can only abolish the requirements for feathers by producing a bird without them.

*Mr Morris:* There is in fact a strain of chickens at the University of California without feathers; they come that way naturally. Hill and Abbott have done a certain amount of work with them and, among other things, have shown that the percentage requirement for sulphur-containing amino acids is indeed lower in these naked chicken for a given rate of live weight net gain than in normal chickens. An answer is thus found to the problem but, unfortunately, these naked chickens only grow at about half the rate of normal chickens, which makes it rather inconvenient to use them for determining amino acid needs.

*Professor G. F. Combs (Maryland):* There are many complications, particularly with regard to availability measurements. The direct assay appears to be the best at the present time; and merits further evaluation.

From some of our recent studies we have accumulated much information which was difficult to interpret until we recognized the need to consider the true intakes of the amino acids. The alternative, of course, is to use pure amino acid diets. With multiple regressional analysis approaches, involving growth and feed intake on the one hand and, on the other hand, growth and available amino acids added, equations of quality for particular groups consuming different amounts of the test protein can be formulated. For example, with the corn gluten meal, unbalanced, basal diet used in our lysine work, we found consistently greater variability than with normal diets.

*Dr Miller:* I agree in that we have done exactly the same thing. In bioassays with lysine we find that the best way to display the results is to plot grams of lysine eaten against weight gain for the chicks. For lysine significantly higher answers were thus obtained than by simply

using weight gain or food conversion efficiency. This device does complicate statistical analysis of the results, and in fact we have had two different statisticians on the problem and they have come up with different answers. With methionine we have done the same thing; it didn't matter whether we plotted the results as grams of methionine eaten or as food conversion efficiency.

Another point I would like to make. I would now like to revert to Professor Brown's comments about the definition of availability. I have added nothing that is not implied in the measurement of quality by the biological value or NPV tests. In measuring the NPV with the rat or with the chick, account has been taken of loss of value due to digestibility—losses of value of amino acids at any stage up to the final utilization—be it in the intestinal tract or be it at the cerebral level. I agree there is no evidence of unavailability at the cerebral level but this is implicit both in the biological value tests and in our own growth assays. All I have done is to give a definition which allows for all possibilities and does not restrict the definition merely to digestibility. For example, I think that a very significant proportion of amino acid (and of limiting amino acid) is probably lost in the intestinal tract due to bacterial de-amination, and this would certainly be included as a loss of digestibility.

# 5

# EVALUATION OF AMINO ACID AND PROTEIN REQUIREMENTS OF POULTRY

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*Synopsis*

Before attempting to state specific amino acid or protein requirements of poultry, consideration must be given to other factors such as the energy content of the diet, temperature, various stress conditions, and strain of bird. These factors all influence feed intake and hence the level of dietary protein required for optimum performance.

Another important consideration is protein or amino acid availability. Most of the work dealing with amino acid requirements of poultry has been done with purified or semi-purified diets. Problems are encountered in using the data so obtained because there are differences in amino acid availability and imbalance between the practical and the purified diets. More work is required on the availability of amino acids in practical feedstuffs before proper use can be made of the currently accepted amino acid requirement values.

Both the efficiency of protein utilization and the effect of diet on carcass composition influence the amino acid and protein requirements of poultry. As the calorie : protein ratio is lowered, protein utilization decreases and at the same time carcasses containing more lean to fat are obtained. Thus economics of production as well as desired composition of product will influence choice of dietary protein or amino acid levels.

Further work in this field should be directed towards the determination of requirement sunder well-defined conditions. The results should then be used with discretion when formulating practical diets.

## *Introduction*

IN discussing protein and amino acid requirements of poultry it is first necessary to define the parameters within which the work is being conducted. It is well known that a number of factors such as energy content of the diet, temperature and various stress conditions will alter feed intake and hence influence the level of dietary protein required for optimum performance. Differences in production and feed intake of heavy- versus light-weight layers can also influence the level of protein necessary to obtain efficient production. Similarly the protein requirement of fast-growing broiler replacement females cannot be directly

compared with that of the slower-growing pullet chicks of egg production strains. Good evidence also exists to suggest that there are differences in protein requirements attributable to strains. Differences in requirement values appearing in the literature can, for the most part, be explained on the basis of certain of the above-mentioned factors.

It is not possible to discuss protein requirements of poultry without at the same time discussing amino acid requirements. In fact, amino acid rather than protein requirements should be the primary consideration. Much work has been done to determine the essential amino acid requirements for the growing chicken. In order to gain precision in amino acid levels in the diets it has been necessary to use purified or

TABLE 1

*Amino acid requirements for the growing chicken*

| Amino acid | Per cent of diet | | Per cent of protein | |
|---|---|---|---|---|
| | 1 | 2 | 1 | 2 |
| L-Arginine | 1·10 | 1·20 | 6·21 | 6·0 |
| L-Histidine | 0·30 | 0·3 | 1·70 | 1·5 |
| L-Lysine | 1·12 | 1·0 | 6·33 | 5·0 |
| L-Tyrosine | 0·63 | 0·7 | 3·56 | 3·5 |
| L-Tryptophane | 0·23 | 0·2 | 1·27 | 1·0 |
| L-Phenylalanine | 0·68 | 0·7 | 3·84 | 3·5 |
| DL-Methionine | 0·45 | 0·45 | 3·11 | 2·25 |
| L-Cystine | 0·35 | 0·35 | 1·98 | 1·75 |
| *L-Threonine* | *0·65* | *0·6* | *3·67* | *3·0* |
| L-Leucine | 1·20 | 1·4 | 6·78 | 7·0 |
| L-Isoleucine | 0·80 | 0·6 | 4·52 | 3·0 |
| L-Valine | 0·82 | 0·8 | 4·64 | 4·0 |
| Glycine | 1·60 | 1·0 | 9·04 | 5·0 |
| L-Glutamic acid | 12·00 | — | — | — |
| L-Proline | 1·00 | — | — | — |
| N×6·25 | 17·69 | 20·0 | — | — |

1. Values of Dean and Scott (1965)
2. United States National Research Council (1960)

semi-purified rations for much of this work. Thus problems exist in extrapolating such data for use in practical rations chiefly because of differences in amino acid availability and balance in practical and purified diets. The latest amino acid requirement figures published for the growing chicken are those of Dean and Scott (1965) (Table 1). Gains of 15 grams per chick per day were obtained between 7 and 13 days of age using a synthetic amino acid diet. These gains compared quite favourably with gains obtained on casein-amino acid and maize-soya bean rations. Expressed as a percentage of the diet, the amino acid requirement values reported by the Illinois workers agree, in general, with those of the United States National Research Council (NRC) values. However, if the amino acid levels are expressed as a percentage of the protein in the diet then it is found that the values of

Dean and Scott are higher for most of the amino acids than the NRC values. These higher values for the Illinois diet reflect a better amino acid balance and availability than envisaged by the NRC for practical type diets.

Much discussion has arisen as to whether essential amino acid requirements should be expressed as a percentage of the diet or as a percentage of the protein. Since amino acids are the building blocks of protein then it would appear logical that they be present in relation to the amount of protein present in diets otherwise adequate in energy. Although various reports have appeared suggesting that the amino acid requirements of chickens decrease with age there is no good information to substantiate the notion that this decline is any faster than the decrease in protein requirement. Several workers have reported that specific amino acids involved in feather formation are required in amounts out of proportion to the level of protein in a diet. However, such a condition exists only during the period of rapid feather growth in the young chick and in general does not alter the concept of amino acid balance in relation to the protein level in a diet.

In suggesting that amino acids must be balanced with respect to the level of protein in a ration it should be pointed out that this is only necessary up to the level of protein that supplies optimum essential amino acid intakes in diets adequate in energy. If the protein level is increased beyond this point then it is not necessary to increase the levels of essential amino acids in proportion to the increase in protein content of the diet. For example, in the diet of Dean and Scott (1965) the protein equivalent was around 17%. If the amino acid levels suggested by the Illinois workers are correct then in formulating 20 or 24% protein diets for starting chicks or broilers the essential amino acids present in the ration should be calculated on the basis of a 17% protein diet, rather than the 20 or 24% diet. This is assuming that the energy level of the practical diets would be the same as that of Dean and Scott so that similar amino acid intakes would be achieved.

In formulating practical diets one must contend with the problem of amino acid availability as well as amino acid balances that are not optimal. Thus it is usually not possible to derive amino acid requirement values that will remain constant as a percentage of the total dietary protein for practical diets. However, if availability values of amino acids were known for the various feed ingredients then the concept of essential amino acids being required as a percentage of available protein or amino acids would be valid. In practical rations, energy content would have to be considered along with amino acid availability and balance and thus in order to ensure the necessary intake of essential amino acids an optimum calorie : available amino acid ratio would have to be taken into account.

While it is true that proper amino acid balance is difficult to achieve in practical rations, and thus one would have to contend with slight

amino acid imbalances, in the majority of practical rations the detrimental effects of an imbalance are almost negligible with protein levels that are optimal for the type of poultry being fed. For example, work in our laboratory with hydrolysed feather meal has shown this product to be not only poorly digested but also extremely imbalanced with respect to the amino acid requirements for poultry. As can be seen in Table 2, substituting feather meal for soya bean meal, markedly

TABLE 2

*Replacing soya bean meal with feather meal in chicken starting diets*

| Per cent dietary protein | Feather meal replacing soya bean meal | Average weight gain (0-4 weeks) gm. |
|---|---|---|
| 15·6 | — | 195 |
| ,, | 3% | 80 |
| ,, | 6% | 71 |
| 19·5 | — | 282 |
| ,, | 3% | 280 |
| ,, | 6% | 264 |
| 23·3 | — | 322 |
| ,, | 3% | 310 |
| ,, | 6% | 320 |

Sibbald, Slinger and Pepper (1962)

reduced growth of chicks on a low protein diet but was a satisfactory substitute at higher levels of protein. By using some feather meal in place of soya bean meal the amino acid balance of the ration was altered and hence the ratio of essential amino acids to total protein was changed (e.g. methionine was reduced). At the lower protein level this change in amino acid balance is enough to result in essential amino acid deficiencies while at the higher protein level the levels of essential amino acids are apparently still high enough to meet the chick's requirements.

Another major point to consider in discussing protein and amino acid requirements is the digestibility of the protein or amino acids. Work in our laboratory has shown that meat meal and feather meal are relatively poor protein supplements when used as the sole source of added protein in a ration. However, when meat meal was supplemented with the essential amino acids in which it is deficient, results equal to those with soya bean meal plus methionine were obtained (Table 3). On the other hand, supplementing feather meal with the essential amino acids in which it is deficient only partially overcame its growth depressing effect (Table 4). It may thus be concluded that meat meal is well digested but has a poor amino acid balance (as compared with the requirement of the chicken) while feather meal protein not only has a poor amino acid balance but is also poorly digested.

Knowing the digestibility of various proteins is very helpful in formulating diets that will more precisely meet the bird's requirements for protein and amino acids. Combs and associates have considered protein digestibilities in their work with linear programming (Combs, Milligan & Martin, 1963; Combs & Nicholson, 1964). They have

TABLE 3

*Amino acid supplementation of meat meal when used as the sole source of protein in 14% protein diets*

| Treatment | Average 3 week weight g. |
|---|---|
| Soya bean meal+0·15% DL-Methionine | 179 |
| Meat meal | 95 |
| Soya bean meal+0·15% DL-methionine +calcium and phosphorus to equal levels found in meat diet | 164 |
| Meat meal—amino acid to equal Soya bean meal | 167 |

Summers, Slinger and Ashton (1964)

assumed the protein digestibility of corn and soya bean meal to be 100% and have related the protein digestibility of other feedstuffs to these ingredients. From the protein digestibility figures they have calculated amino acid digestibility values assuming that all amino acids are equally digested. Work in progress in our laboratories indicates

TABLE 4

*Amino acid supplementation of feather meal protein when used in 14% protein diets*

| Treatment | Average 3 week weight g. |
|---|---|
| Soya bean meal+0·15% DL-methionine | 183 |
| Feather meal | 73 |
| Feather meal+amino acids to equal soya bean meal | 108 |
| Feather meal level increased by 45% | 76 |
| Feather meal+A.A. to equal soya bean meal +supplements of the 10 E.A.A. at 45% of the level found in feather meal | 105 |

Summers, Slinger and Ashton (1965*b*)

that this may not be a valid assumption. We are determining amino acid digestibility values for various feedstuffs using the rat. The results to date suggest that all amino acids are not equally well digested—at least in certain wheat by-products (Table 5). It appears that the amino acid balance in practical diets can be improved by working with amino acid rather than protein digestibility figures. It is our

intention to repeat some of the digestibility work using colostomized birds in the hope of obtaining a good correlation between the chick and the rat. If such a correlation is found it would simplify the work by permitting the use of the rat as an aid to chick feed formulation. On the other hand, since only a relatively small percentage of the total

TABLE 5

*Amino acid digestibility of wheat by-products*

| Amino acid | Bran digestibility % | Middlings digestibility % |
|---|---|---|
| Threonine | 79 | 79 |
| Methionine | 74 | 79 |
| Valine | 78 | 81 |
| Isoleucine | 75 | 81 |
| Leucine | 78 | 86 |
| Phenylalanine | 80 | 87 |
| Lysine | 72 | 79 |
| Histidine | 86 | 90 |
| Arginine | 86 | 91 |

Olsen, Summers and Slinger (unpublished data)

nitrogen in the urine of the chicken is amino acid nitrogen it may well be possible to use non-operated chicks for amino acid digestibility studies in which the errors are only minor.

The shortcomings of some of the amino acid values are recognized; for example tryptophan is destroyed on hydrolysis and other amino

TABLE 6

*Amino acid supplementation of wheat bran and middlings*

| | 10 day weight gain g. | Nitrogen digestibility % | NPU |
|---|---|---|---|
| Soya bean meal+amino acids | 33·1 | 90·9 | 68·7 |
| Bran | 10·6 | 80·8 | 50·7 |
| Bran+2·26% amino acids | 28·2 | 81·1 | 60·4 |
| Bran+1·13% amino acids | 23·2 | 82·3 | 58·4 |
| Midds | 22·5 | | |
| Midds+2·02% amino acids | 35·2 | | |
| Midds+1·01% amino acids | 27·2 | | |

Olsen, Summers and Slinger (unpublished data)

acids, especially cystine and methionine, can also be partially lost. The balance method that is being used may also be criticized on the basis that the method of determining metabolic faecal nitrogen and the presence of bacterial amino acids can introduce errors in the digestibility figures obtained. However, feeding trials suggest that the digestibility values obtained may be fairly accurate (Table 6). Supplementing bran

with levels of essential amino acids necessary to equal the requirement of the rat resulted in a marked improvement in weight gain and NPU. Reducing this level of supplemental amino acids by one half reduced the weight gain and the NPU values slightly. A similar situation is shown for the weight gain of the rats fed middlings. The lower gain of the bran-fed rats may well be explained by the inability of the animals to consume enough of the high fibre bran ration.

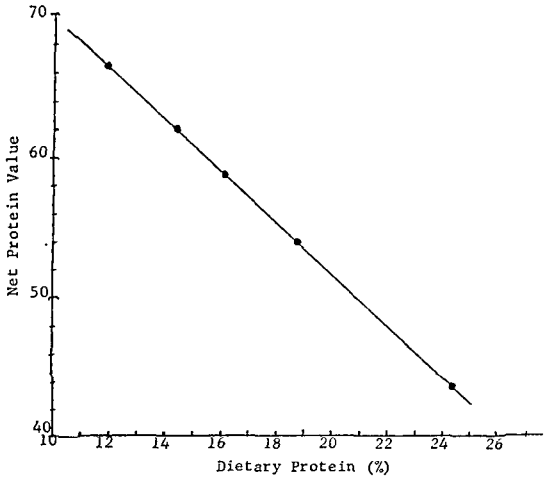


FIG. 1. Effect of level of dietary protein on NPU.
(Summers and Fisher 1961)

If necessary other methods will be investigated with the hope of arriving at some method that will give reliable estimates of amino acid availability. Amino acid availability values should be additive, and if reliable values can be obtained for feedstuffs the achievement of proper amino acid balance in practical diets can be greatly aided.

Even if satisfactory amino acid digestibility data are obtained there are still many other factors to consider in assessing the optimum protein or amino acid levels in diets. It has been demonstrated many times that as the level of dietary protein is increased the percentage nitrogen retention decreases. An example of this is shown in Fig. 1. In this work and in most other work demonstrating this point the calorie/protein ratio of the ration has decreased as the level of protein increased. If, however, the calorie/protein ratio is held constant as the level of dietary protein is increased, similar protein utilization values are

obtained and carcass composition remains constant (Table 7). If the energy level is kept constant in the diet as the protein level is increased, a decrease in protein utilization and a leaner carcass is obtained

TABLE 7

*Nitrogen utilization and carcass composition of birds fed various levels of dietary protein in diets of similar calorie/protein ratio*

| Treatment kcal. M.E./g. | Protein % | Calorie/protein ratio | Average 3 week weight g. | N.P.U. % | Carcass protein (dry weight basis) % |
|---|---|---|---|---|---|
| 2·50 | 18 | 63 | 197 | 51 | 60·5 |
| 2·78 | 22 | 57 | 222 | 52 | 61·0 |
| 3·33 | 26 | 58 | 235 | 51 | 58·6 |

Summers, Slinger, Sibbald and Pepper (1963)

TABLE 8

*Nitrogen utilization and carcass composition of birds fed various levels of protein in iso-caloric diets*

| Treatment kcal. M.E./g. | Protein % | Average 3 week weight g. | NPU % | Carcass protein (dry weight basis) % |
|---|---|---|---|---|
| 2·50 | 10 | 132 | 66 | 53·4 |
| 2·50 | 14 | 179 | 62 | 56·6 |
| 2·50 | 18 | 197 | 51 | 60·5 |
| 2·50 | 22 | 210 | 48 | 64·4 |
| 2·50 | 26 | 220 | 39 | 63·4 |

Summers, Slinger, Sibbald and Pepper (1963)

TABLE 9

*Nitrogen utilization and carcass composition of birds fed increasing levels of energy in iso-nitrogenous diets*

| Treatment kcal M.E./g. | Protein % | Average 3 week weight g. | NPU % | Carcass protein (dry weight basis) % |
|---|---|---|---|---|
| 2·50 | 18 | 197 | 51 | 60·5 |
| 2·78 | 18 | 205 | 54 | 57·5 |
| 3·05 | 18 | 201 | 58 | 55·3 |
| 3·33 | 18 | 202 | 61 | 52·8 |

Summers, Slinger, Sibbald and Pepper (1963)

(Table 8). Keeping the level of protein constant and increasing the level of energy results in increased nitrogen retention and decreased carcass protein (Table 9). In feeding Junior Broilers practical maize-soya bean diets similar weights were obtained over a six-week period

with protein levels ranging from 20 to 26%. However, similar carcass composition was not obtained (Table 10). If birds of similar carcass composition are desired it will be necessary to feed different levels of protein and energy to males and females and perhaps also to different strains. From Table 11 it can be seen that to achieve similar carcass composition under these conditions the males would have to be fed a

TABLE 10

*Effect of dietary protein level on carcass composition of 6-week-old broilers*

| Level of dietary protein | Carcass fat (dry weight basis) | |
|---|---|---|
| | ♂ | ♀ |
| % | % | % |
| 20 | 23·7 | 29·8 |
| 22 | 23·0 | 28·3 |
| 24 | 22·1 | 26·4 |
| 26 | 20·4 | 22·6 |

Summers, Slinger and Ashton (1965*a*)

diet of around 20% protein while the females would require a diet containing about 26% protein. The results presented in the last five tables clearly demonstrate that protein utilization and carcass composition are readily influenced by the calorie/protein ratio of the diet.

Amino acid requirements for laying hens have also been determined. Here again problems are encountered in interpreting amino acid and

TABLE 11

*Effect of level of protein on egg production and egg size*

| Protein level | Average egg production HDB | Feed consumption per 100 birds/day | Average egg weight |
|---|---|---|---|
| % | % | lb | g |
| 12 | 60·5 | 21·5 | 55·0 |
| 14 | 75·8 | 23·4 | 57·4 |
| 16 | 74·2 | 23·4 | 58·5 |
| 18 | 74·1 | 23·1 | 59·8 |

Slinger, Summers and Pepper (unpublished data)
* *HDB=Hen day basis*

protein requirement data for use in practical diets. While egg production has been maintained on low protein diets, maximum egg size has not been achieved. In Table 11 are shown the results of work conducted in our laboratory where four different protein levels were fed to hens for a 12-month period. Although the higher protein level resulted in the largest eggs the question arises as to the economic significance of producing the larger egg. The level of protein in laying hens'

diets must also be considered in relation to the fatty liver syndrome which has created problems in some areas. Work in our laboratory has shown that the level of dietary protein can influence the degree of fat infiltration of the liver (Table 12). However, the energy level was

TABLE 12

*Influence of level of protein on percentage liver fat*

| Level of protein % | Average egg production HDB* % | Feed/100 birds per day lb | Liver fat (dry weight basis) % |
|---|---|---|---|
| 13 | 76·4 | 23·7 | 49·3 |
| 15 | 77·0 | 23·6 | 40·2 |
| 17 | 78·0 | 23·6 | 38·2 |

Slinger, Summers and Pepper (unpublished data)
* HDB=Hen day basis

reduced in these diets as the level of protein increased and thus the calorie/protein ratio was lowered. Thus here again we see the fallacy of speaking in terms of the effect of dietary protein without taking into account the level of energy and hence absolute protein intake.

TABLE 13

(a) *Influence of protein level and amino acid supplementation on weight gain of large white turkeys from 16-26 weeks of age*

| Period (weeks) | | | Amino acid addition | | Average weight | Weight gain | Feed/gain |
|---|---|---|---|---|---|---|---|
| 16-20 | 20-24 | 24-26 | | | 26 weeks | 16-26 weeks | 16-26 weeks |
| level of protein | | | lysine | methionine | kg | kg | |
| % | % | % | % | % | | | |
| 18 | 16 | 14 | — | — | 13·3 | 5·5 | 5·61 |
| 18 | 16 | 14 | 0·05 | — | 13·4 | 5·5 | 5·62 |
| 18 | 16 | 14 | 0·1 | — | 13·3 | 5·7 | 5·50 |
| 18 | 16 | 14 | 0·05 | ·05 | 13·3 | 5·6 | 5·51 |
| 16 | 14 | 12 | — | — | 13·4 | 5·6 | 5·54 |
| 16 | 14 | 12 | 0·05 | — | 13·3 | 5·4 | 5·66 |
| 16 | 14 | 12 | 0·1 | — | 13·4 | 5·6 | 5·54 |
| 16 | 14 | 12 | 0·05 | ·05 | 13·6 | 5·7 | 5·57 |

(b) *Lysine values of diets (determined)*

| Protein level | Lysine % of protein |
|---|---|
| 12 | 3·8 |
| 14 | 4·0 |
| 16 | 4·2 |
| 18 | 4·7 |

Summers, Slinger and Pepper (unpublished)

Another important factor to take into account when considering protein and amino acid requirements is the strain of bird employed. Work in our laboratory (Table 13) has shown that fast-growing large

white turkeys require around 4% of lysine as a percentage of the protein during the growing-finishing period as compared to a value of 5% as recommended by Balloun (1962). The birds in our experiment weighed approximately 2·8 kg more at 24 weeks than did those in Balloun's experiment. One possible explanation for the lower lysine requirement in our experiment is that the faster growing bird can utilize protein more efficiently. It is also axiomatic that the faster growing birds require a smaller amount of their total protein intake for maintenance.

Another factor to consider in amino acid supplementation of diets is the interpretation of results from short-term studies. Possible interactions between vitamin $B_{12}$, choline and methionine, in a practical ration, were investigated in a 4-week experiment with commercial egg-production pullets. In this experiment (Table 14) only methionine

TABLE 14

*The interrelationships between vitamin $B_{12}$, choline and methionine in practical chick starting diets*

| Supplementation | | | | |
|---|---|---|---|---|
| Methionine 500 mg/kg | $B_{12}$ 0·0132 mg/kg | Choline 330 mg/kg | Average 4-week weight gm | Feed/gain |
| − | − | − | 269 | 2·24 |
| − | − | + | 264 | 2·25 |
| − | + | − | 270 | 2·22 |
| − | + | + | 272 | 2·18 |
| + | − | − | 283 | 2·14 |
| + | − | + | 286 | 2·09 |
| + | + | − | 280 | 2·15 |
| + | + | + | 282 | 2·17 |

By analysis the basal diet contained 0·36% methionine, 1980 mg of choline per kilo and 0·0030 mg of vitamin $B_{12}$ per kilo of diet.

Slinger, Pepper and Summers (unpublished)

produced a significant response. The question which may logically be asked is 'Should one recommend methionine supplementation of such a diet during the starting period?' In order to answer this question we need complete life-cycle experiments to study the influence of methionine supplementation in the starting period on subsequent performance during the growing and laying periods. Similar long-term studies are required on level of dietary protein and amino acid supplementation of diets for all classes of poultry throughout their complete life-cycles.

In considering protein and amino requirements of poultry attention should always be paid to economic as well as physiologic requirements. Diets which are nutritionally optimal may not always be economically sound.

Further work in the area should have as its goal the determination

of requirements for more well-defined stocks for shorter periods of time and these should be integrated based upon long-term feeding studies. Conditions under which requirements are determined should be defined and the results used with discretion when formulating practical diets.

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# 6

# QUALITY TESTS FOR PROTEIN CONCENTRATE FOODS

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*Synopsis*

Though much remains to be done, considerable progress has been made in developing tests for predicting quality in protein concentrates used in poultry diets. Chemically determined available lysine (ALV) has given excellent correlations with the results of animal feeding experiments for supplements of animal origin but a corresponding test for plant protein materials has not as yet achieved universal acceptance. Microbiological evaluations of protein quality and of the availability of various essential amino acids appear promising. Tests based on nitrogen solubility, digestibility, and dye binding, as well as tests designed to examine special features of the particular protein, such as the urease test for the detection of underheating in soya bean meals, have proved satisfactory although frequently the ranges of test samples are small and the correlations demonstrated only fair.

*Introduction*

ATTEMPTS were being made to evaluate protein feeding stuffs by biological methods in the early years of this century using feeding trials of a more or less empirical kind. Much of this work was of dubious value owing to the unsuitability of the basal diets used and to ignorance of the vitamin, mineral, and energy requirements of various classes of livestock. Also, these biological tests were time-consuming and not amenable to routine use. As a result manufacturers and consumers came to rely upon the simple and rapid measurement of nitrogen content as a guide to the economic value although it was early realized that other factors were also important in judging the nutritive value of proteins. It is against this background that recent work aimed at discovering more informative rapid tests of protein quality should be examined.

Because so many factors influence the quality of protein the prospect of discovering a single and comparatively simple test might seem to be hopeless. It is however possible that for a given class of protein feedingstuff one or two factors may be of over-riding importance. For example it has been repeatedly shown that ideal conditions of heating are needed

to produce optimum quality in soya bean meal and it may be that a test which correctly indicates the extent of heating will provide all the information necessary for the commercial grading of soya bean meal samples. It must be remembered that a single protein feedingstuff is unlikely ever to be called upon to provide all the protein in a commercial ration for poultry and consequently the nature of the accompanying materials must be taken into consideration when assessing the nutritive value. Furthermore the value of a protein food will depend upon the need that it is to be called upon to meet. A protein suitable for a mature laying hen may not necessarily be adequate for a fast-growing broiler chicken. Thus to be serviceable any predictive test should indicate the nutritive value of the material under a given set of conditions, and the more diverse these conditions are the more generally useful the test will be. As the technique of total amino acid analysis improves on the one hand and our knowledge of the animal's requirements for particular amino acids in its diet becomes clearer there is no doubt that such a test will make an important contribution in quality predictions. However, availability of amino acids rather than the total level present is the more useful criterion, though total amino acid analysis can expose a serious deficiency in a protein. Friedman (1958) concluded that amino acid analysis could not be used to predict protein quality because other factors such as digestibility and antitryptic substances must be considered.

In this review only laboratory tests of protein quality will be considered. Microbiological methods are included as these are comparatively rapid and suitable for routine use but no mention will be made of procedures which involve the use of animals such as rats, chicks or pigs. The primary function of protein concentrates is to provide protein. Other constituents of these feeding meals are incidental and therefore tests of nutritive value associated with the vitamin, mineral or energy content will not be considered here.

### *Measurements of Digestibility, Nitrogen Solubility and 'True' Protein*

Having briefly outlined the difficulties facing research workers in this field, attempts to resolve them may be considered. Not unnaturally the first attempts to improve upon a simple nitrogen analysis were based on measurements of digestibility, nitrogen solubility and 'true' protein. Each of these attempts to exclude from consideration fractions of the total nitrogen which are likely to be physiologically of lesser importance. In 1935 Almquist, Stokstad and Halbrook proposed the Protein Quality Index (PQI) as a means of grading feedingstuffs. The PQI is a factor obtained from four chemically determined values, comprising copper-precipitable, hot-water-soluble, phosphotungstic acid-precipitable, and pepsin-indigestible fractions of the total nitrogen, and in arriving at the

final expression an attempt was made to give due weight to the contribution which each was considered to make to the total nutritive value of the material. In this and in a subsequent publication (Almquist, 1941) good correlations were reported between PQI and chick growth rate for various animal by-products. Evans and St John (1945) reported significant correlations between the PQI of soya bean meals, cottonseed meals and peas, and a measurement of the supplementary value in cereal-based diets for chicks—the Gross Protein Value (GPV). The PQI was one of the tests chosen for study in a collaborative investigation, organized by the Agricultural Research Council, which began in 1955, and in the first Progress Report it was shown that there were good correlations between PQI and GPV for a range of 13 whale meals and 17 fish meals, but not for 19 meat and meat/bone meals (Boyne, Carpenter & Woodham, 1961). In a subsequent publication by Barnes and Woodham (1963), removal of three non-typical fish meals from the previously reported range resulted in a lowering of the correlation coefficient from 0·74 to 0·52. Estimation of 'true' protein and pepsin-soluble nitrogen in the ranges of meat, fish, and whale meals used in the ARC collaborative investigation yielded no significant correlations with either GPV or with Net Protein Utilisation (NPU) determined by the rat method of Miller and Bender (1955). In the paper already cited Evans and St John reported the application to soya bean meals of a nitrogen fractionation procedure devised by Lund and Sandstrom (1943), finding that nitrogen solubility in 0·2% KOH correlated with GPV. In the ARC collaborative investigation it was found that all fractions correlated with GPV to some extent for cottonseed meals, but no correlation was found for soya bean, sunflower seed or groundnut meals (Boyne *et al.*, 1961). Barnes and Woodham (1963) suggested that the failure to confirm the findings of Evans and St John was due to the use of a more comprehensive range of soya bean meals. Using rat growth tests Olcott and Fontaine (1942) obtained a correlation with the percentage of the total nitrogen of cottonseed meals which was soluble in 3% NaCl solution. Lyman, Chang and Couch (1953) did not find a similar correlation for chicks but found instead a satisfactory one using 0·02N NaOH. This was confirmed for the series of 17 cottonseed meals used in the ARC collaborative trial (Boyne *et al.*, 1961; Barnes & Woodham, 1963). A nitrogen solubility of 75% indicates a cottonseed meal of the highest quality for non-ruminant feeding and a figure of 70% has been widely accepted in the U.S.A. as the critical one below which cottonseed meals should be diverted to ruminants.

Nutritive value must obviously be affected by digestibility to some extent and considerable work has been carried out on *in vitro* digestibility determinations (e.g. Gehrt, Caldwell & Elmslie 1955; Bondi & Birk 1955). However proteins are not always completely split by these methods and the results are difficult to relate to *in vivo* digesti-

bilities. Failure to obtain correlation between *in vitro* pepsin digestibilities and animal feeding experiments for fish meals has led recently to an attempt to establish at an international level an improved and standardized pepsin digestibility test. It had been noted that tests carried out in Germany and Austria used much less pepsin than the amount commonly used elsewhere and it was then discovered that some thirty years ago a decimal error had crept into the literature. In the recent work the level of pepsin was reduced to one hundredth or one thousandth of the quantity specified in the method recommended by the Association of Official Agricultural Chemists (1960). Good correlations were obtained between results of the modified test and chemically determined available lysine values, but there were indications that the sensitivity of the test may be such that it indicates differences between meals which are indistinguishable in animal tests (Olley, 1964). The low pepsin strength method measures rate of digestion rather than extent of digestion (Lovern, 1965) and it has been repeatedly emphasized that pepsin solubilization alone is no index of quality. Water or acid solubility of the protein must be taken into account.

### *Microbiological Methods for evaluating Protein Quality*

Microorganisms may be used to study the quality of entire protein, or, by virtue of differing requirements for particular amino acids, they may be used in amino acid availability estimations. This second application will be considered in the next section.

The three organisms which have been chiefly used for investigations into protein quality are *Streptococcus faecalis* (Halevy & Grossowicz, 1953); *Streptococcus zymogenes* (Ford, 1960); and *Tetrahymena pyriformis W* (Stott, Smith & Rosen, 1963). Bunyan and Price (1960) obtained a good correlation between the growth of *Strep. faecalis* and Net Protein Utilization for whale meals. Boyne *et al.* (1961) confirmed this, finding good correlations also with GPV for whale meals. They noted too that *Strep. zymogenes* gave good correlations with NPU and GPV for whale meals but with NPU only for meat meals ($r=0{\cdot}73$). *T. pyriformis* yielded a correlation with GPV for meat and whale meals. Waterworth (1964) found good correlations between relative nutritive values (RNV) determined with *Strep. zymogenes* and both the NPU and GPV ranges reported by Boyne *et al.* (1961) for the same samples of fish, whale and meat meals. Stott, Smith and Rosen (1963) described a simplification of the *T. pyriformis* assay (Rosen & Fernell, 1956) applied to 93 protein foodstuffs.

### *Measurements of Amino Acid Availability*

The biggest single factor affecting the nutritive value of protein feedingstuffs is the amino acid composition and more especially the

availability of particular amino acids. Apart from biological methods, the description of which is inappropriate here, amino acid availability may be measured microbiologically or chemically. The chemical estimation of available lysine (ALV) is now firmly established as a routine chemical procedure used widely in research laboratories everywhere, and its importance cannot be overestimated. The method relies upon the reaction of 1-fluoro-2:4-dinitrobenzene (FDNB) with the unbound $\epsilon$-amino groups of lysine and gives a measure of the proportion of the total lysine which is available for use in the metabolic processes of the animal. This application to animal protein concentrates by Carpenter and Ellinger in 1955 of the reaction used by Sanger (1945) in his fundamental studies on the structure of insulin, subsequently modified by Carpenter (1960) provides a rapid test, the results of which have been shown to give extremely good correlations with chick growth tests (Carpenter, Ellinger, Munro & Rolfe, 1957; Boyne *et al.*, 1961). The method has been used recently to evaluate animal protein concentrates in the absence of biological checks in order to study the possibility of allocating a mean figure applicable to the majority of samples of a given type (Pritchard, McLarnon and McGillivray, 1964). Wide ranges were found and average figures could be applied to only about 50% of the samples in each class. This is in agreement with the findings of Duckworth, Woodham and McDonald (1961). Bunyan and Woodham (1964) reported the use of the ALV as a screening test for the selection of fish meals of differing nutritive value for an experiment designed to test the validity of applying to pigs conclusions drawn from chick experiments, and Laksesvela (1958) has found good correlations between ALV and chick growth estimations for a series of experimentally heated herring meals. Olley and Watson (1961) used available lysine content of a number of fish meals in a study of the effects of preservation, putrefaction and drying methods. Parallel digestibility studies revealed that high digestibility is not always to be associated with a high available lysine content.

The work mentioned so far concerns only animal protein concentrates. Certain difficulties arise when materials high in carbohydrate are examined and this has resulted in a comparative dearth of reports on the available lysine content of plant protein foods. Prolonged heating in the presence of carbohydrate, inevitable during the hydrolysis stage of the FDNB test, leads to disappearance of the $N^{\epsilon}$-dinitrophenyllysine. Losses may be of the order of 25 to 30% in the case of groundnut and soya bean meals. Allowance can be made for these losses after carrying out recovery checks using pure $N^{\epsilon}$-dinitrophenyllysine heated under identical conditions, but the large corrections required do not inspire confidence in the result. It is probably justifiable to use the method to distinguish between plant proteins which differ fairly widely in nutritive value, but absolute figures should be quoted with appropriate reservations. The use of the method for plant

protein evaluation has been reported by Carpenter and March (1961)—groundnut biscuit; Butterworth and Fox (1963)—coconut meal; Jones, Livingston and Cadenhead (1965)—soya bean meal; Anantharaman and Carpenter (1965)—groundnut flour. Erbersdorfer and Zucker (1964) have claimed that their modified method allows the estimation of ALV in mixed feeds high in carbohydrate providing that the lysine level is greater than 1%. Modifications introduced specifically to make the method more suitable for use on plant proteins have been suggested by Rao, Carter and Frampton (1963) who separated the $N^{\epsilon}$-dinitrophenyllysine from other components of the hydrolysis mixture on an ion-exchange column, and by Mauron and Bujard (1963) who guanidinated with *O*-methylisourea prior to hydrolysis in order to convert the lysine with free epsilon amino groups into homoarginine which is stable to acid hydrolysis. Good results were obtained by the guanidination procedure for milk powders and the results for groundnut and soya bean meals appeared promising. Further reports are awaited with interest. Paper chromatography has been used to separate the hydrolysis products (Baliga, Bayliss & Lyman, 1959). With cottonseed meals this was found to give results which correlated satisfactorily with protein quality evaluations using rat protein repletion tests.

Micro-organisms have been used to measure the availability of some amino acids. The availability of lysine and of methionine has been measured using the organism *Tetrahymena pyriformis W*. A simplified method has been described by Stott *et al.* (1963) and modifications have been reported by the same authors in Bunyan and Woodham (1964) where results are reported for three fish meals.

The organism *Streptococcus zymogenes* has been shown to possess an absolute requirement for methionine, leucine, isoleucine, arginine, histidine, tryptophan and valine, and Ford (1962) has described a method for measuring the availability of these amino acids. Lysine, threonine and phenylalanine are not indispensable for the organism and consequently it cannot be used for estimating their availability. Ford made the point that much more complete information is needed from animal tests. From the evidence presented there is a suggestion that the availability to the rat of the amino acids measured in whale and fish meals must parallel closely the availability to *Strep. zymogenes*. It may be noted also that fair correlations were found between GPV (chick) and available methionine (*Strep. zymogenes*) and between GPV and available tryptophan ($r$=0·66 and 0·63 respectively). Waterworth (1964) using Ford's method with certain modifications found good correlations between the chick GPV reported by Boyne *et al.* (1961) and the availabilities of arginine, histidine, leucine, isoleucine, methionine, valine and tryptophan for the same range of whale meals; with available histidine ($r$=0·88) and several others ($r$ about 0·6) for the same range of fish meals; and with available methionine ($r$=0·94) and available histidine and leucine ($r$ about 0·7) for the same range of meat

meals. In the whale meal series and to some extent in the fish meals the availability of each of the amino acids correlated with the availability of each of the others leading to the suggestion that a portion of the entire protein may be unavailable. Ford (1964) found that enzymic predigestion and fine grinding of test samples increased the values obtained for fish and whale meals, and comparing the results with those of Bunyan and Price (1960) he noted that the microbiological assay figures for available methionine and tryptophan and the chemically determined ALV were all closely correlated with digestibility and biological value. Recently in a series of meat, fish and whale meals which included some of those used in the ARC collaborative work, Miller, Carpenter, Morgan and Boyne (1965) have found good agreement between available methionine determined by *Strep. zymogenes* and available methionine determined by a chick assay ($r=0{\cdot}93$). These authors noted that the microbiological results were affected by the concentration and activity of the papain used for predigestion of the samples. Ford (1965) has submitted enzymically digested fish and whale meals to Sephadex-gel filtration and has noted that the digests of the poorer quality materials contained relatively more material of large molecular size which proved a poor source of microbiologically and chemically determined available amino acids. This approach would seem to merit further attention.

### *Dye Absorption Methods*

In 1927 Chapman, Greenberg, and Schmidt titrated proteins such as casein and gelatin with six different dyes and established a quantitative relationship between the extent of dye binding and the free basic groups of arginine, lysine and histidine. Rawlins and Schmidt (1929) found a stoichiometric relationship between protein level and absorption of basic dyes. Fraenkel-Conrat and Cooper (1944) devised methods for determining the total acidic and basic groups of proteins based upon their ability to combine with dyes in buffered acidic or alcoholic solution. Orange G was found especially satisfactory and in 1954 Udy used this to study wheat protein fractions. Frölich (1954) successfully used cresol red to detect under- and over-heating in soya bean meals and his results were confirmed by Olomucki and Bornstein (1960) and by Ascarelli and Gestetner (1962). Correlations between GPV and Orange G binding were observed for eight whale meals and eight fish meals, the correlation coefficients being 0·72 and 0·87 respectively, but no correlations were noted in a range of 11 groundnut meals (Boyne *et al.*, 1961). Bunyan and Price (1960) found good correlations between the extent of Orange G binding and NPU measured with rats by the method of Miller and Bender (1955) for ranges of meat and whale meals. Choppe and Kratzer (1963) have used Orange G for the evaluation of a range of 20 meat meals finding

significant correlations ($r=0{\cdot}68$) with the results of a chick growth test when the dye binding values were combined with estimates of nitrogen solubility in hot water.

### *Tests for Toxic Constituents*

Under this heading are included specific tests for materials known to be harmful and whose presence may be suspected in the case of particular types of protein food. Gossypol is known to reduce the nutritive value of cottonseed meal and evidence has been adduced which suggests that it may act by rendering lysine unavailable (Lyman & Baliga, 1958). The commercial production of glandless, and therefore gossypol-free cottonseed meal which has been shown to be of high nutritive value (Johnston & Watts, 1964) may eventually resolve the problem. Similarly the presence of aflatoxin in groundnut meal may cease to be a problem when efficient methods of harvesting and storing the groundnuts become universal (Spensley, 1963). Morrison, Sabry and Middleton (1962) noted the toxic effects for rats of fish flours which had been extracted with chlorinated solvents, and this should be borne in mind where there is the possibility that such processes may have been used.

### *Miscellaneous Tests*

Meat by-products are notoriously variable in nutritive value and also in the nature of the raw materials used in their preparation. Large proportions of connective tissue are undesirable. Recent work has suggested that the availability of lysine is in indirect proportion to the amount of connective tissue present in cuts of raw meat (Dvorak & Vognarova, 1965). Hydroxyproline is a characteristic constituent of collagen and Eastoe and Long (1960) suggested that it might be used as a measure of quality in meat products. Summers and Fisher (1962) attributed poor quality in four meat meals to the high collagen content as indicated by hydroxyproline analysis. Ascarelli and Gestetner (1962) on the other hand found no correlation between hydroxyproline content and rat NPU for fish meals, and Woodham (unpublished results) similarly found no correlation between hydroxyproline content and chick GPV for a series of 31 meat and meat/bone meals. Grant (1964) has published a method for estimating hydroxyproline by the Technicon Auto Analyser.

Measurements of antitryptic or urease activity were found useful by Ascarelli and Gestetner (1962) for distinguishing between underheated and properly heated soya bean meals but Boyne *et al.* (1961) failed to predict nutritive value in a series of soya bean meals with the urease test when it was used in conjunction with solubility measurements which might be expected to indicate overheating. Medium urease values

did not apparently indicate the meals of highest nutritive value as had been suggested by earlier workers (Croston, Smith & Cowan, 1955).

Miller and Carpenter (1964) determined total sulphur in a series of seven meat, fish and whale meals but found no correlation with rat NPU probably because cystine and methionine together accounted for less than two-thirds of the total sulphur in five of the samples. Similar results were reported for meat and whale meals by Boyne *et al.* (1961).

An interesting development reported recently (Johnston & Watts, 1965) is the prediction of quality in glandless cottonseed meals by means of their infra-red spectra. Changes in the spectrum were noted corresponding to the differing nutritive values of the samples.

## *Discussion*

Various tests have been mentioned all of which, in the hands of some workers, have been found useful for predicting nutritive value in one or more of the classes of protein concentrate commonly incorporated in poultry diets. None so far is acceptable to all workers for all protein materials and it may well be that no single simple test will ever emerge as a panacea. The nearest approach to such a test is probably the chemical estimation of available lysine, accepted widely now as a good indicator of quality for animal by-products, and there is little doubt that in time a modification acceptable to all will extend the test to plant proteins. Provision of lysine in available form is undoubtedly a desirable characteristic in a protein concentrate, but obviously the test will not be useful in predicting the value of a supplement to a particular basal diet which is, for example, low in the sulphur amino acids. Undoubtedly a useful addition to the nutritionist's armoury would be a similar test for available methionine and cystine. Microbiological evaluations have exposed to view the possibility of non-availability in other essential amino acids and have underlined the fact that total amino acid analysis can be very misleading. One of the most significant of recent developments has been the emergence of microbiological procedures as very promising tools in this type of investigation. Recent collaborative work between microbiologists has shown that attention to detail can result in considerable reductions in the extent of inter-laboratory variability, but much remains to be done especially to ascertain the extent to which results for the availability of particular amino acids for microorganisms may be applied to large animals.

Though the ideal is a single rapid test such as the highly successful solubility measurement which appears to be quite adequate for the assessment of cottonseed meals, it seems that in general, combinations of tests are more likely to provide us with useful information. The successful linking of Orange G binding and hot water solubility has been mentioned and another useful combination of tests is embodied in the Protein Quality Index.

Tests such as the urease and aflatoxin tests are specific for particular types of concentrate and their inclusion in this paper emphasizes the fact that any protein quality evaluation method must be related to the material under investigation. Such tests are further arguments against the likelihood of the eventual emergence of a single simple test for all materials. The search for new tests should not therefore be allowed to slacken and the infra-red spectra study (Johnston & Watts, 1965) may well be capable of extension to other plant proteins.

Doubts concerning the validity of particular tests frequently arise when small numbers of test samples are used. In work of this type the number of samples should be as large as possible and even when highly significant correlations with biological tests are noted for series of say 20 samples, conclusions should still be drawn only with considerable reservations. One or two samples deviating inexplicably from a general pattern may not affect the correlation coefficient very much but they do tend to destroy confidence in the test and prejudice its adoption by others. On the other hand, perfect correlations cannot be expected because of the inherent variability of the biological criteria which must be used.

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# 7

# EVALUATION OF CEREAL PROTEINS

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*Synopsis*

The nutritive value of the crude protein in samples of cereal intended for poultry feeding, depends on the concentrations of individual amino acids in that crude protein. These concentrations in turn depend on the relative proportions of the individual proteins making up the crude protein of the cereal. Reference is made to the influence of factors such as crop husbandry and genetical background on these proportions in samples of wheat and maize.

A study with young chicks is described in which all-cereal diets were fed *ad libitum*. The concentration of protein in each cereal was about the same namely around 10%. Experiments indicated that the value of cereal proteins for promoting growth was in descending order oats, barley, wheat or maize, findings not wholly unexpected in view of published average amino-acid compositions of these cereal proteins and estimates of the amino acid requirements of growing chicks. The findings are compared with other published work.

## *Introduction*

THE greater part of the energy provided in poultry rations is derived from the carbohydrate of cereals which, with few exceptions, form the basis of all rations for farm livestock. As a result of this predominance, cereals also play an important part in providing some of the other nutrients normally required to support satisfactory production. In consequence much research has been directed to devising rations providing adequate supplies of balanced nutrients and using the maximum proportions of cereals—which are normally among the least costly components of the ration—and the minimum of more expensive supplementary materials. The amount of supplementary protein concentrate required in turn depends on the type of production envisaged as well as on the concentration of protein in the cereal component and the balance of amino acids in that protein.

## *Amino Acid Balance and the Protein Content of Individual Cereals*

It is now well established that climate, soil type, fertilizer treatment and genetical factors all influence the final crude-protein content of

cereals (see reviews by Duckworth, 1952; Woodham, 1963). However, the proportions of essential amino acids in a cereal do not necessarily remain unchanged as the crude-protein content alters. For example, there would appear, for wheat, to be an inverse relationship between the proportion of lysine in the crude protein, and crude-protein concentration in the cereal (Schweigert, 1948; McElroy, Clandinin, Lobay & Pethybridge, 1949; Price, 1950; Gunthardt & McGinnis, 1957; Lawrence, Day, Huey & Lee, 1958; McDermott & Pace, 1960; Simmonds, 1962; Hepburn & Bradley, 1965). This probably applies also for maize (Sauberlich, Chang & Salmon, 1953; Miller, Aurand & Flach, 1950; Eggert, Brinegar & Anderson, 1953) and barley (McElroy *et al.*, 1949). No corresponding evidence was found for oats. Even where such relationships have been found however, it cannot be assumed that they are completely linear, for Lawrence *et al.* (1958) have found for wheat an inverse linear relationship with lysine up to 13·5% protein but not above this value. Some of the amino acids, less important from a nutritional standpoint, such as glutamic acid, are found in greater proportions in cereals of high protein content (McDermott & Pace, 1960; Simmonds, 1962).

Such variation in the amino acid proportions in cereal protein seems to arise because an alteration in total crude protein content is the result of an alteration in the content of constituent proteins, each of which may differ in amino acid composition and in the extent to which it occurs in cereals of different nitrogen content. For example, there is a higher proportion of the protein zein in high-protein maize than in low-protein maize (Hansen, Brimhall & Sprague, 1946; Frey, 1951) and it has long been known that for animal feeding zein is poorly balanced in its content of amino acids because it contains very low concentrations of lysine and tryptophan. Thus in high-protein maize one might expect a balance of amino acids which is of less value nutritionally than is the case in a low-protein maize.

Similarly for wheat, it has been shown in chromatographic studies by Simmonds (1962) that high concentrations of total protein are associated with low proportions of soluble albumins and globulins which are relatively good sources of lysine, and high proportions of the insoluble gluten proteins gliadin and glutenin, which are particularly poor sources of lysine. Apparently the proportion of endosperm proteins, largely gliadin and glutenin, tend to be lower in wheats of low crude protein content than in wheats of high crude protein content. Furthermore, the endosperm proteins from low-protein wheats tend to be associated with a higher concentration of lysine than do those from high-protein wheats (Morris, Alexander & Pascoe, 1945; Lawrence *et al.*, 1958) possibly because of a higher proportion of the lysine-rich soluble protein (McDermott & Pace, 1960). It is also possible that the gliadin, which has a very low content of lysine, may be present in smaller amounts in the endosperm of low-protein wheats.

relative values of cereal proteins different species of animals have been used and the manner in which the protein has been fed has varied. In some cases the cereals have been given alone and in others together with a common basal mixture containing protein concentrate. This latter procedure may have tended to mask differences between cereal proteins. On several occasions one or other of the cereal proteins has been shown to be superior to the others tested—either in digestibility or in utilization of digested nitrogen or both—but the overall impression remained that there was no appreciable difference in nutritive value of the protein. In the case of poultry, however, this is not what one would expect after inspecting Tables of average amino acid composition for cereal proteins, because the lysine, which is generally a limiting essential amino acid in diets based on cereals, is more plentiful in oat protein than in the proteins of barley, wheat or maize. Fig. 1 shows the requirements of the chick, laying hen and turkey poult (Agricultural Research Council; Committee on the Nutrient Requirements of Farm Livestock, 1963) expressed as a percentage of the crude protein contents frequently used in diets for each type. For comparison the concentration is given of each amino acid in certain cereal proteins (de Man &

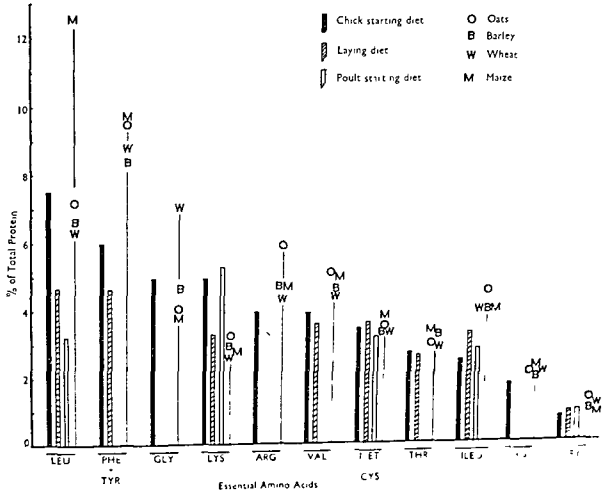


FIG. 1. Requirements of the starting chick, laying hen and turkey poult as a percentage of dietary protein taken as 20%, 15% and 28% respectively, compared with the amino acid content of oats, barley, wheat and maize.

The general impression gained then from the majority of studies involving fractionation of the nitrogenous compounds in wheat and maize is that separated components differ in the relative proportions of their amino acids and that the relative amounts of these components may vary in samples of different nitrogen content. Unfortunately it would appear that the proteins which are increased most readily in the seed by improved husbandry are frequently the ones with relatively low concentrations of certain important essential amino acids, in particular lysine. An interesting paper by Wolfe and Fowden (1957) indicates that in the case of maize at least, genetical selection offers some hope for improving the nutritive value of cereal protein. These authors found that in seven samples of East African maize containing similar nitrogen contents between 1·32 and 1·65%, the lysine in the protein ranged from as low as 3·5 to as high as 6·3%. These divergent values were associated with genetic differences.

At this point it is perhaps worth considering that the alteration in the proportion of lysine in wheat protein which occurs with increasing nitrogen content of the sample, is relatively small compared with the alteration in nitrogen content itself. Gunthardt and McGinnis in 1957 found that in contrasting samples of wheat containing 1·7 and 2·7% nitrogen the lysine in the protein was 3·60 and 3·26% respectively. They considered this difference of one-tenth to be of little importance in practice. More recently Hepburn and Bradley (1965) have shown that in wheat an increase in nitrogen content from 2 to 3% was associated with alterations in individual amino acid concentrations in the protein of less than one-tenth. There seems little doubt that the contribution of any particular amino acid made by wheat is determined primarily by the amount of protein it contains, a high-protein wheat always containing greater amounts of a particular amino acid than a wheat having a considerably lower protein content. As a result of their studies of samples of hard wheat and despite the inverse relationship found between important amino acids like lysine and the crude protein content, Hepburn and Bradley (1965) came to the conclusion that the constancy of amino acid values between varieties and between types served to increase confidence in the use of representative analyses for predicting the contribution of amino acids made by wheat in diets. This may apply equally well to maize, barley and oats, although the findings of Wolfe and Fowden (1957) in regard to maize would always have to be borne in mind till further information became available.

### *Amino Acid Balance in Individual Cereals*

In compounding rations for livestock it is frequently desirable to know whether cereal proteins are freely interchangeable from the protein quality point of view and many studies have been carried out which have a bearing on this question. In experiments to assess the

relative values of cereal proteins different species of animals have been used and the manner in which the protein has been fed has varied. In some cases the cereals have been given alone and in others together with a common basal mixture containing protein concentrate. This latter procedure may have tended to mask differences between cereal proteins. On several occasions one or other of the cereal proteins has been shown to be superior to the others tested—either in digestibility or in utilization of digested nitrogen or both—but the overall impression remained that there was no appreciable difference in nutritive value of the protein. In the case of poultry, however, this is not what one would expect after inspecting Tables of average amino acid composition for cereal proteins, because the lysine, which is generally a limiting essential amino acid in diets based on cereals, is more plentiful in oat protein than in the proteins of barley, wheat or maize. Fig. 1 shows the requirements of the chick, laying hen and turkey poult (Agricultural Research Council; Committee on the Nutrient Requirements of Farm Livestock, 1963) expressed as a percentage of the crude protein contents frequently used in diets for each type. For comparison the concentration is given of each amino acid in certain cereal proteins (de Man &

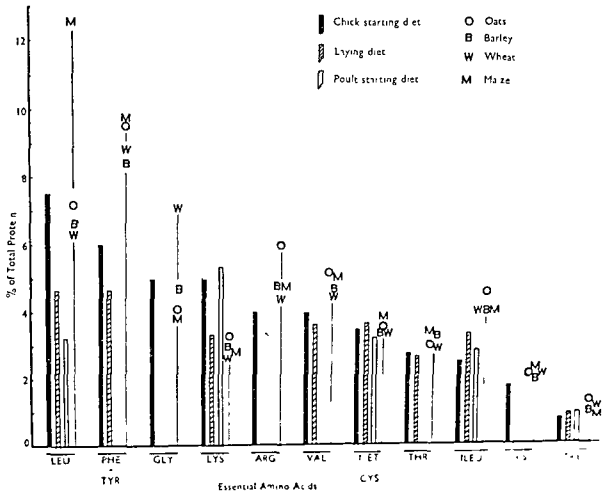


FIG. 1. Requirements of the starting chick, laying hen and turkey poult as a percentage of dietary protein taken as 20%, 15% and 28% respectively, compared with the amino acid content of oats, barley, wheat and maize.

Zwiep, 1955). It is obvious from Fig. 1 that in cereal diets, lysine and the sulphur amino acids are the ones likely to limit production. Oats appear to contain the most favourable protein for growing birds whilst maize or a mixture of maize and oats might have the most favourable amino acid composition for laying hens. It is of interest here to recall that Shaw and Nightall (1955) were able to keep laying hens in full production for prolonged periods on diets of mixed cereals containing no more than 11% crude protein providing the birds had access to runs where presumably they were able to forage for sufficient animal

TABLE 1

*Experiments 1 and 2. Percentage composition of diets*

| Ingredient | Wheat diet | Oat diet | Barley diet | Maize diet |
|---|---|---|---|---|
| Wheat, coarsely ground | 64·5 | — | — | — |
| Oats, Sussex-ground | — | 70·5 | — | — |
| Barley, ground | — | — | 70·5 | — |
| Maize, ground | — | — | — | 69·0 |
| Oat feed | 17·0 | — | 9·5 | 20·0 |
| Wheat gluten | 5·0 | 5·0 | 5·0 | 5·0 |
| Dried yeast | 1·0 | 1·0 | 1·0 | 1·0 |
| Vitamin supplement* | 1·5 | 1·5 | 1·5 | 1·5 |
| Choline supplement† | 0·52 | 0·5 | 0·5 | 0·5 |
| Vitamin $B_{12}$ supplement‡ | 0·0 | 0·02 | 0·02 | 0·02 |
| $Ca_3(PO_4)_2$ | 2·0 | 2·0 | 2·0 | 2·0 |
| $CaCO_3$ | 0·5 | 0·5 | 0·5 | 0·5 |
| NaCl | 0·5 | 0·5 | 0·5 | 0·5 |
| Maize starch | 7·5 | 18·5 | 9·0 | — |
| | 100·0 | 100·0 | 100·0 | 100·0 |

* Commercial preparation containing, per 10 lb, 4,000,000 I.U. vitamin A, 2,000,000 I.U. cholecalciferol, 2 g riboflavin, 2 mg vitamin $B_{12}$, 10 g nicotinic acid, 2 g pantothenic acid, 1 g vitamin E, 25 g Fe, 36 g Mn, 7 g Cu, 3 g Co, 10 g I, 23 g Zn (V. W. Eves & Co. Ltd, Ilford, Essex, 1959).

† Commercial preparation containing 25% choline chloride (V. W. Eves & Co. Ltd, Ilford, Essex).

‡ Commercial preparation containing 15 μg vitamin $B_{12}$/g (Distillers Co. Ltd, Speke, Liverpool).

or grass protein to make up small deficiencies in lysine or the sulphur amino acids.

Studies with poultry up to 1960 (Van Landingham, Clark & Schneider, 1954; Carpenter & Clegg, 1957) relied mainly on nitrogen balance techniques which give an indirect measure of nitrogen retention In a study carried out at the Rowett Institute some years ago (Davidson, Mathieson & Williams, 1962) it was decided to use carcass-analysis techniques to obtain a more direct measure of the nitrogen laid down in young chicks between one and four weeks of age.

In order to ensure that an excess of protein would not obscure amino acid deficiencies in the protein tested, a protein content of around 11% and a metabolizable energy (ME) content of 2·6 Mcal/kg

were chosen for the diets which are shown in Table 1. This protein level could not be achieved with cereals alone and so of the 11% protein, some 4% was provided by wheat gluten, which, being a cereal protein itself with an amino acid pattern similar to that of cereals, was not expected to interfere greatly in the comparison. The fibre content was raised to 6% in each diet by adding oat feed. The indigestible organic matter of each diet was calculated to be about 22%. Maize starch added to each diet brought the ME concentration to approximately the same figure and the resultant CP/ME ratio (g/Mcal) of 44 was well below the critical level of about 60.

Results were obtained from two experiments feeding the diets given in Table 1 and a third in which the diet formulae were somewhat modified in case the protein contributions from the oat feed added to balance the diets in indigestible organic matter were not available. Fig. 2, including data from all three experiments, shows that for a given protein intake the protein gain in the tissues was greatest when oats was the cereal in the ration. Barley protein was less efficient and wheat or maize proteins least efficient. Statistical analysis showed that when the results from the first two experiments were combined, percentage retentions of dietary protein on the oats, barley, maize and wheat rations were 30·1, 27·5, 24·1 and 24·7 respectively (S.E. of differences $=\pm 0{\cdot}59$) whereas in the third experiment, with the somewhat modified

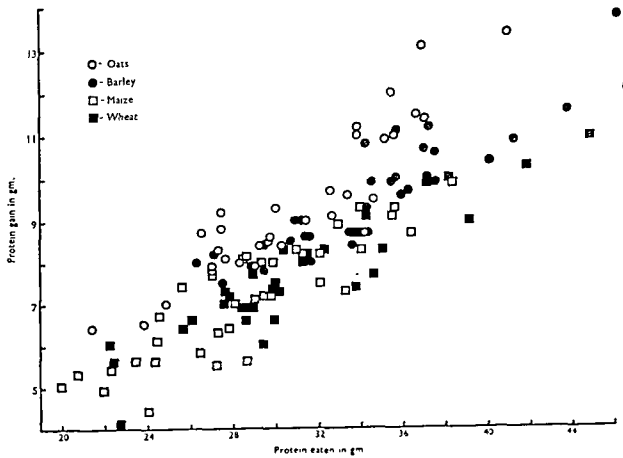


FIG. 2. Relationship between protein gain and dietary protein eaten on the four cereal diets.

Carpenter, K. J. & Clegg, M. K. (1957). The relative value of three cereals as protein sources for growing chicken. *Br. J. Nutr.*, 11: 358-364.
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Frey, K. J. (1951). The interrelationships of protein and amino acids in corn. *Cereal Chem.*, 28: 123-132.
Gunthardt, H. & McGinnis, J. (1957). Effect of nitrogen fertilization on amino acids in whole wheat. *J. Nutr.*, 61: 167-176.
Hansen, D. W., Brimhall, B. & Sprague, G. F. (1946). Relationship of zein to the total protein in corn. *Cereal Chem.*, 23: 329-335.
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Morris, V. H., Alexander, T. L. & Pascoe, E. D. (1945). Studies of the composition of the wheat kernel. I. Distribution of ash and protein in center sections. *Cereal Chem.*, 22: 351-361.
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Simmonds, D. H. (1962). Variations in the amino acid composition of Australian wheats and flours. *Cereal Chem.*, 39: 445-455.
Van Landingham, A. H., Clark, T. B. & Schneider, B. H. (1945). Protein utilization and supplementary relationship of protein concentrates in basal diets of cereal grains and cereal by-products for growing chickens. *Poult. Sci.*, 24: 105-111.
Wolfe, M. & Fowden, L. (1957). Composition of the protein of whole maize seeds. *Cereal Chem.*, 34: 286-295.
Woodham, A. A. (1963). The feeding value of cereals. *J. Nat. Inst. Agric. Bot.*, 9: 402-408.

rations and different cereal samples, percentage retentions were 30·5, 28·5, 26·5 and 24·7 respectively (S.E. of differences $=\pm 0{\cdot}76$).

The conclusion reached at the end of this study was that the value of cereal proteins for promoting growth in young chicks was in the order oats > barley > wheat or maize thus giving support to the view that oats is a highly reliable cereal if somewhat lacking in energy content.

Butterworth (1962) has reported studies also carried out at the Rowett Institute in which chicks between two and four weeks of age were used to compare the quality of proteins in wheat, barley and maize. In these studies the proportion of dietary nitrogen retained on the barley diet was no greater than on the wheat or maize diets either by balance experiment or by direct carcass analysis. It may be that the presence in the basal diet of a large amount of wheat offals having a protein relatively rich in lysine reduced any effect arising from differences in quality between the test proteins. Feeding 7-week-old pullets at a protein level between 10 and 11% of which just over half was contributed by test cereals, Carpenter and Clegg (1957) found that barley protein was inferior to oats and maize. This was so whether the major protein supplement was white fish meal or groundnut meal. Here again the amino acid balance in oats appears to have been more advantageous than that of barley for growth. The equality of maize and oats in this study is most interesting when one recalls the work of Wolfe and Fowden (1957) who found, within the normal range of protein contents, individual varieties of maize which had a much higher proportion of lysine than the average.

Perhaps the present position in regard to the relative nutritive values of cereal proteins might be summed up as follows. There is evidence both from chemical analysis and from feeding trials with growing chicks that of the cereals normally used in the United Kingdom oats provides protein of the greatest nutritive value. In compounding diets for broilers, however, the beneficial balance of amino acids in oats would have to be set against the high content of indigestible organic matter, a factor that would tend to reduce food intake owing to reduced acceptability if the content of oats were too high, but there might be considerable value in feeding diets high in oats to replacement pullets for which fast growth is not of prime importance. There is no information about the laying hen, but from chemical analysis it would appear that oats or oats and maize mixtures might require minimum supplementation with expensive protein concentrates.

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## DISCUSSION ON PART II

*Professor A. S. Parkes:* I sympathize with your encounter with statisticians, two of whom produced different answers—you were lucky they didn't each produce alternative answers. (Laughter).

*Dr M. J. Head (Battersea):* The story of the three papers in the last session, and indeed that of the morning session as well, was the need to evaluate proteins on the basis of their relative nutritive values so that they may be used to their best advantage. This has been emphasized recently by the serious world shortage of protein feedstuffs, and a better knowledge of the value of a protein in filling a particular biological need would obviously help to stretch the available supplies a little further.

The scientific barriers to a complete biological evaluation of protein seem to be formidable, hence one of several alternatives has to be accepted. It is thought that the amino acid pattern of the protein is, from a nutritional point of view, its most important attribute. However, each acid has a separate availability factor, so that the first possibility is the determination of the availability of each important amino acid in each main class of ingredient. The second, and more practical, possibility, is to use a biological assessment of the value of the whole protein as it exists for nutritional use, while the third possibility is to rely on a chemical measurement having a high correlation with a biological parameter.

At the moment, there is clearly only one practical course open to the manufacturer of feed, namely to use a quick chemical method, and in particular the available lysine technique of Carpenter. Many are doing this with success. The major limitation of the method is that in the assay of vegetable protein a large correction factor, due to the interference by carbohydrates is necessary. There are, however, signs that this obstacle may shortly be overcome.

The aim must be a simple chemical or physical method for the assessment of the protein value of an ingredient, or better still of a mixed feed, that ranks test materials in the correct order.

In his paper, Dr Summers has explained the niceties of the amino acid nutritional picture. In particular he says that practical diets are compounded on the basis of the total amino acid composition of the several proteins, although each of these amino acids has in fact an availability factor attached to it. The data presented also indicated that essential amino acid requirements are commonly supplied and their imbalances rendered of no consequence by an oversupply of protein. The shortages and imbalances that can occur in practical diets are, however, well illustrated by the elegant experiments on the influence of diet on carcass and egg composition.

The assessment of relative values of proteins in practical feeds is a large and rapidly moving problem and this makes the able review by Dr Woodham the more serviceable. The conclusion must be that the available lysine technique of Carpenter is the best one to use on animal proteins, but unfortunately it does not yet reach high standards of reproducibility and recovery when applied to vegetable proteins. There are however specific methods for measuring the protein quality of cottonseed and soya bean meals while infra-red absorption mentioned by Woodham may possibly provide the rapid all-purpose technique which everyone is wanting. Microbiological methods too have their devotees who hope to find among them the universal laboratory method of assessment of protein quality.

One interesting general point ought to be mentioned, namely that the biological criterion for the chemical or microbiological techniques, is always one of growth. This is a cause of concern to those who will use, rather than derive, the quality scores. In the context of this Conference is the absence of protein quality data for egg production of serious consequence? Although egg production is nutritionally more efficient than growth, a broad assessment of protein quality based on growth studies is likely to be applicable to the laying hen.

An equally intriguing point is the nutritional efficiency in the dietary of poultry of preformed proteins as distinct from amino acids. The relative ratings of rations with or without high levels of synthetic amino acids should be compared with either the results of chemical or microbiological laboratory tests of protein quality.

Cereal proteins may provide up to 50% of the protein in poultry rations, hence small differences in value between the proteins of the various cereals could be quite important. Again the basis for assessment has been chick growth and there is little published work on hens. Dr Davidson's conclusion that a relatively high valuation can be put on oat protein is of interest, particularly in relation to rearing laying or breeding stock where, as the result of practical experience, the same conclusion has been reached.

The relatively high crude protein (i.e. $N \times 6{\cdot}25$) contents of cereals grown with heavy application of nitrogenous fertilizer poses a difficult problem for nutritionists. The additional nitrogen-containing substances of the cereal are largely non-proteinaceous and there is little information about their nutritive value. The whole question of non-protein nitrogen utilization by poultry awaits thorough investigation.

The other side to the story of protein quality determination is the great virtue it can have to the compounder, and therefore in the end to the farmer, in enabling the best value for money to be obtained. The number of types of fish meal available in Britain as feed ingredients is very large, and while the nutritional worth of some is half that of others, prices reflect content of crude protein more closely than content of biologically useful protein. Data obtained on the biological

quality of a protein source, may well be too late for the gristing of the parcel from which it came. Such information however allows the compounder to build up a picture of the quality of meals from different sources, so that the buyer may be guided in his choice of the source of purchase.

It is becoming increasingly apparent that the greatest influence on the quality of a protein ingredient is the processing the original product undergoes after collection. Processing may include solvent extraction, pressing, rendering or heating to dryness to avoid spoilage. As all these processes are more or less controllable, there is good reason to hope that once the potentialities of the situation are better understood the general quality of protein meals will rise.

One barrier to the proper exploitation of scientific advances in this field is still the farmers' uncritical acceptance of protein content rather than protein quality as a test of value. Work is urgently needed to break down this barrier.

*Professor R. A. Morton (Liverpool):* May I ask, what glandless cotton seed meal is?

*Dr. A. A. Woodham (Aberdeen):* I'm not a botanist, but I understand that the toxic constituent in cotton seed meal, the substance gossypol, is entirely confined to these portions of the seed called the glands. By producing a glandless cotton seed we have eliminated this toxicity. I understand that enough is now being produced for quite extensive feeding trials and I think eventually it will be a commercial proposition. It has already been shown to be nutritionally equivalent to ordinary cotton seed meal.

*Dr C. Calet (Jouy-en-Josas):* May I first say that the very interesting paper of Dr Summers confirms what I said this morning. I would like however to return to a particular point concerning the effect of the protein level of the diet on protein efficiency. Dr Summers told us that good weight gains could be obtained with proteins of poor biological value by raising the protein level of the diet. We have obtained similar results for weight gain and we have also studied the effect of these diets on feed efficiency (see Table below):

| | Low biological value proteins | | | High biological value proteins | |
|---|---|---|---|---|---|
| protein level | 19% | 26% | 35% | 19% | 26% |
| weight gain (g) | 101 | 122 | 131 | 121 | 132 |
| feed intake (g) | 200 | 206 | 204 | 217 | 214 |
| feed efficiency | 1·99 | 1·68 | 1·53 | 1·79 | 1·62 |

From these results, it appears that the diets containing proteins of poor biological value but having a high protein level (26 and 35%) produce the same weight gain at 4 weeks of age as the diets with proteins of high biological value and lower protein level (19 and 26% respectively).

However, in respect of the same weight gain, the low value proteins always give the best feed efficiencies. This is due to the effect of the protein level on feed intake (see Calet *et al., Comptes Rendus Acad Sci.*, Paris, 1964, 258; 3104-3106).

*Professor J. D. Summers (University of Guelph, Canada):* Most of our work has been on net protein utilization. Neither the protein nor the amino acid balance changed, so that I cannot answer your point. My only query is whether, with a poor biological value, you are obtaining the same carcass composition. My guess would be that, with an unbalanced protein of poor biological value, you gain the same weight but probably have a fatter bird.

*Dr C. Calet:* We have actually studied the body composition of the animals when the protein level of the diet increases.

(i) The number of calories per gram of carcass decreased and the decrease was higher with proteins of high biological value than with proteins of low biological value.

(ii) In all cases the fat content was higher with fish meal than with peanut meal (which were the protein sources studied). However when the protein consumption increased, the fat content of the carcass increased with peanut meal and decreased with fish meal.

The apparent discrepancy between (i) and (ii) arises from the difference between the amount of protein retained and the amount of water retained.

*Professor G. F. Combs (University of Maryland):* Inasmuch as I am going to spend considerable time tomorrow discussing this subject, perhaps I should only mention briefly at this time what we have found. When the protein level of the diet of chicks is raised above the requirements of essential amino acids, improved feed efficiency is consistently observed. This occurs when the increased protein level is or is not in balance with respect to amino acid quality. This effect of protein level, which does not appear to be due to specific amino acid make-up, appears to be greater in a cold than in a hot environment. I would expect from our work that birds growing at the same rate but having poor quality protein would have a better feed efficiency and a higher body protein and a lower body fat content than chicks fed lower levels of better quality protein.

*Professor Brown (Belfast):* I wonder whether some of these high levels of protein give rise to excessive deamination of amino acids, the products being used for energy. It is extremely difficult to account for these results without at the same time having figures for energetic efficiency. How far can food conversion efficiency be used to assess protein quality with any degree of accuracy when diets so widely different in protein content are being compared?

*Dr G. D. Rosen (London):* Bearing in mind that we have now had about a decade of research work on the measurement by chemical means of 'available' lysine in protein concentrates, can any information

or guidance be offered on the relation between the 'available' lysine values and economic value of the animal protein concentrates?

*Dr R. F. Gordon (Houghton):* In this work on quick laboratory tests our ultimate aim remains to improve the economic utilisation of proteins. The link here is simply that we hope available lysine values will provide the manufacturer with a yardstick which he can use in preference to a simple nitrogen determination. He can then utilise his raw materials more efficiently and sell his product more cheaply.

*Dr Rosen:* But the question remains: by how much can the price of the feeds be reduced? Or by how much should the valuation per ton of the concentrates be raised or lowered according to 'available' lysine content?

*Dr Gordon:* That is a good point. In academic circles we are not quite so closely in contact with economics as we might be. But we do appreciate and keep on saying that all or nearly all the commercial feeds do in fact waste protein. Thus chick starter diets and pig grower rations have been shown experimentally to contain more protein than they really need to do. To make allowance for the poor quality proteins (of ground nut and so on), the compounder has to put in 2 or 3% more protein than he would otherwise do. With a more accurate method of evaluation, such as the available lysine promises to be, the excess of protein could be saved, with corresponding economic gain.

*Professor Morton (Liverpool):* At what level of protein in the diet does specific dynamic action begin to be serious in poultry?

*Dr Calet:* We have done some experiments on this subject and I think specific dynamic action is very important if the diet is unbalanced in amino acids. With a very good balance of amino acids in a diet for growing chicks dynamic specific action is very low.

*Mr R. Hill (Royal Veterinary College, University of London):* I would like to ask Dr Woodham to clarify a point on the interpretation of GPV. He showed a slide with a large range of values obtained for individual feeding stuffs. These results are very useful for distinguishing between a good sample and a bad sample of a particular feeding stuff. Does it follow that a poor quality fish meal and a good quality cotton-seed meal with identical GPVs give the same growth rate with chicks?

*Dr Woodham:* Under the conditions of the GPV test, certainly, they will but I have no information regarding performance under widely differing practical conditions. We did in fact produce correlations between GPV and ALV for meat, fish and whale meals, and noted that while the meat and fish meals could be accommodated on the same regression line, whale meals fell on a slightly different one. This would suggest that GPV is measuring somewhat different things in the various series and the same situation could hold for fish and cottonseed meals. The GPV is, however, only a means to an end and we hope that ALV will provide us with a numerical factor which will enable us to group

concentrates according to their quality and independently of their nature.

*Mr W. R. Muir (Glaxo Laboratories):* The tacit assumption is made that the feed compounder—the buyer—should test the quality of his protein concentrates, and this, by Jove, he hasn't been doing up to the present time. But surely it should be obligatory on the part of the seller to provide a specification, or on the buyer to demand a product meeting a specification. There are economic advantages in the production of protein concentrates conforming to specific standards. Of course, the products available are not all of top quality. Certain fish meal manufacturers at the moment are selling on the basis of an available lysine test but they are nevertheless charging a price per unit of 1% of protein-crude protein. Surely it ought to be sold at a price per unit of available lysine, assuming that this available lysine test (as most people here seem to think) is the best guide. I personally don't; as I mentioned this morning, there are some fish meals which don't correlate very well. That is the point I particularly wanted to make.

It is interesting how closely the quality of the protein in wheat matches the theoretical requirements for egg production. As there is a world shortage of protein for man could we not mechanically concentrate the protein of wheat and use the surplus starch, along with simple nitrogen compounds, for fermentations that produce our beer. We could then use the yeast to raise the concentration of protein in our poultry foods.

*Mr F. S. D. Brown (Eastern Counties, Ipswich):* It should of course be recognized that the available lysine test has already had quite a profound effect on the quality of proteins which the feed compounder has on offer. I must declare my interest and say straightaway that we are mainly buyers of protein ingredients for compound feeds, and for some years as part of our quality control technique have used the ALV test. It is significant that in recent years the number of samples of animal protein products of lower biological value, particularly meat meals, offered to us has been reduced. It would thus appear that the manufacturers themselves have paid particular attention to processing technique and quality of their raw materials.

*Mr C. J. L. Baker (Ministry of Agriculture, Cambridge):* I was very pleased to find from Dr Davidson's results that barley was running oats a fairly close second. There has been, and I believe still is, a prejudice against the use of barley in quantity in poultry rations. I would like to ask Dr Davidson whether such prejudice is justified or should we be forthright in encouraging the use of more barley in poultry feeds?

*Dr Davidson:* As you say, the experiments I have done indicate that, for the growing chick, barley runs a good second to oats in protein quality. Perhaps one reason why barley is not used so much as maize and wheat is that it is lower in energy content and cereals are used in the main to provide energy in the ration. Barley is a very good cereal

but its energy content is against it. Perhaps there are others here who know more about feeding barley in practice than I do.

*Professor Summers (University of Guelph):* We have done some work feeding barley to laying hens. Excellent production has been obtained where barley has been used as the sole cereal in a soya bean type diet. However, pounds of feed per dozen eggs was quite a bit higher than with a corn, soya bean ration. Where barley can be purchased economically a high percentage can be used in laying diets. One would have to consider the ability of the hens to consume the extra quantity of feed in order to achieve their necessary energy intake. This could be a problem with a light strain of birds or in community type cages where often feed consumption is reduced due to competition at the feed trough. Crumbling of the diet would help to overcome this problem.

*Dr B. R. Taylor (Nottingham):* Would Professor Summers amplify his remarks about the problem of wet droppings when barley is used? We have used experimental rations for layers containing up to 76% of barley, which was thus, the whole cereal component. These diets have been fed to layers in cages where we would be concerned about undue moisture content of the droppings, but we didn't have any problems. This was British barley, with about 9% of crude protein.

*Professor Summers:* I would add that one of the recommendations we give to overcome this wet droppings problem in cages is to add 5 to 10% of oats or barley to the laying ration. The litter will actually contain more water in total, but because of the increased bulk the droppings will cone up better and thus the problem of sloppy droppings is eliminated. On a ration containing all corn and soya bean meal very loose droppings are usually experienced. However, the performance of the birds does not appear to be affected.

*Professor Brown (Belfast):* I would like to ask Dr Woodham, who has wide experience of these chemical and microbiological tests, just how far he thinks we can take them. Should we recommend them to compounders and go so far as to say that determination of lysine alone or methionine alone will be sufficient. I ask this question because I feel that we need a concept which includes utilization as well as availability. Would Dr Woodham agree that, as far as poultry are concerned, we cannot at this juncture dispense with specification of total protein? I say that because it must be obvious to all of us that the hen has a frank requirement for amino nitrogen, and on that basis would he not think that some figure such as a product of utilisation and total amino nitrogen might give a better assessment?

*Dr Woodham:* I think you may be right, Professor Brown, but the point about the available lysine test is that it provides, or we hope it will provide, the manufacturers with a comparatively quick test. When we started this work in 1955 the great cry was for a way to replace the conventional nitrogen analysis with a more informative

test. The only available tests were biological and they took, at the very minimum, two weeks. Even then they were probably not acceptable to the great majority of people. Everyone had his own ideas about biological testing at that time. The advantage of the available lysine test is that it does give us something—not perhaps the whole answer but something useful. It can be carried out in 16 hours in other words, it involves overnight hydrolysis and about three hours' work at the bench the next morning. It gives a useful figure which is certainly better than a nitrogen analysis. We might use other tests equally time-consuming but, in the meantime, I regard available lysine as the best we have and anybody interested in a quick test would do well to try it.

The solubility test I mentioned is obviously ideal for cottonseed meal but, special cases apart the ALV is the one thing to follow up and when we can extend it to vegetable materials, the feed compounders will snap at it. Possibly measurement of infra-red absorption can be adapted on a routine basis. It would be a quick test and if it did expose processing damage (perhaps the most important thing as far as oil seed meals are concerned) we might be able to solve the problem of testing soya bean, sunflower seed and similar meals. In my view the ALV is the best test available and we should use it.

*Professor D. Lewis:* May I carry on from the previous speaker? The ALV test involves an overnight hydrolysis and a three-hour laboratory run the following morning; would not an overnight hydrolysis and a fast amino acid run on a Technicon autoanalyser taking five hours give much more useful information in very little more time?

*Dr Woodham:* It could be so; I haven't any experience of five-hour runs on the machine and I don't know what the resolution is like, but in any case I am not at all happy that a total amino acid analysis, no matter how quick, could usefully replace the determination of ALV.

*Professor D. Lewis:* May I make the point that an independent test can be done on a total feed as opposed to just one on a protein ingredient.

*Dr Woodham:* Undoubtedly this is useful. I would certainly like to have it but I would rather have the available lysine of a principal constituent. The available lysine of the principal protein concentrate, to my mind, is more important than the total amino acid analysis of the complete feed.

*Professor D. Lewis:* Everyone with experience of the ALV test knows—although a beginner might not—that the available lysine measured doesn't include prelysine, which though not important normally might become so if lysine is added as a supplement. The test doesn't separate hydroxylysine nor ornithine. Hydroxylysine can be found where there is some connective tissue and ornithine (from lysine) in heated fish meals. This is not a major criticism of the method, it is merely a cautionary remark on what is indeed a very good method.

*Dr G. D. Rosen (London):* It is noteworthy that whereas there are reports by the hundred of variation in the quality of the protein in concentrated protein feedingstuffs of both animal and vegetable origin, there are in contrast I believe, only three reports mentioned by Dr Davidson comparing the quality of the protein in three or four cereal grains. This is regrettable, since cereal protein can contribute anything from 5 up to 70% of the total in a compounded feed. We recognise however that the low concentration of protein in the various cereal grains is something of a barrier to their evaluation in respect of protein quality. To some extent these handicaps can be overcome since protein quality assays on cereals can be carried out more abundantly, using a protozoan *Tetrahymena pyriformis W.* This organism can be fed the intact proteins in cereals or in compounded livestock and poultry feeds, and it has the same ten essential amino acid requirements as young animals.

Results to date are in general accord with cereal protein assays on higher animals and indeed confirm that the protein of oats is somewhat superior in quality to that of the other common grains, wheat, barley, maize, rice and milo. In addition we have observed striking differences (up to 100%) in the protein quality of individual varieties of barley or wheat or maize, quite apart from well-known differences in protein content. We feel, therefore, that the future may well reveal greater variation in cereal protein quality, be it due to varietal, seasonal, geographical or other influences including fertilizers.

*Dr D. G. A. Guttridge (Spalding):* To me, as a compounder, ALV is fine for rejecting unsatisfactory samples but we sometimes get deliveries that turn out to be average or barely average and we have to use them. On what basis should the resulting calculations be made so as to satisfy the requirement of the stock that is to be fed. Were the estimates of requirement for lysine determined on complete proteins and, if so, how did the element of availability affect this determination? How on earth is confusion avoided if a material is totally available but the requirement has been assessed on material that was perhaps 85% available but assumed to be 100%?

*Dr Miller:* If you are satisfied that a given fish meal has an ALV value of 6 and another has an ALV value of 5 you might use more of the bad one by that proportion.

*D. G. A. Guttridge:* Yes, this is the only way you can do it, presumably.

*Professor J. McGinnis (Washington State University):* I would like to direct a question to Dr Miller. If we find a sample of fish meal that has its available lysine value considerably reduced by processing does this treatment influence availability of any or all of the other amino acids? Or is the damage confined to lysine?

*Dr E. L. Miller:* Well, there is really very little evidence on which to give an answer but Ousterhout (Ousterhout *et al.* (1959) *J. Nutr.* 69:

65-73), I recall, published results for one badly overheated fish meal. Bioassays on that particular sample showed reduced availability for every amino acid studied. In our own chick bioassays we found that decreases in available lysine, were accompanied by roughly similar decreases in available methionine and available isoleucine.

*Dr M. J. Head (Battersea College):* It is important in relation to the point Guttridge made that the various determinations produce not an absolute figure but a ranking order of one ingredient against another. The relationship of ranking to absolute requirement, though fairly important remains yet to be established.

*Dr C. Fisher (Reading):* There is also need to help those who are trying to define the amino acid requirements of the animals themselves. In what terms should we define our rations at the present time, so that the data we obtain should be of the maximum use?

*Professor J. McGinnis (Washington):* I would like to make one additional comment. Even though amino acids other than lysine, perhaps methionine and cystine, are reduced in availability by poor processing, it doesn't necessarily follow that this is going to influence the performance of the animal. The reason for this is that, when the diet is formulated, the margin above the minimal requirement of the animal may be so large that the amount of destruction or the decrease in availability is of no significance. In some work that was done with soya bean meal about 15 years ago we severely damaged it by heating in the autoclave for two hours at 130°C. It looked very much like coffee when it came out. We corrected and supplemented this product with lysine and methionine and, by so doing, completely restored its feeding value to the chick, even though some other damage had undoubtedly taken place. So, to come back to the original question, perhaps if the available lysine test shows a sample of fish meal to be poor, all you have to do is fortify the diet with lysine and to rely on the total analytical results for all the other amino acids (or whichever one is in question).

*A speaker:* At the present price of lysine it would be far cheaper to add a little bit of soya bean meal. (Laughter).

*Dr Miller:* In view of the discussion on differences between cereals, it is of interest that the bioassays carried out by Gupta and others (1958) and by Calhoun and others (1960), to which I referred in my paper, indicate low availability of lysine in maize and wheat, in harmony with Dr Davidson's results. However, the methods of bioassay used were not perfect, and further work on the availability of amino acids in cereals would be highly desirable.

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# 8

# AMINO ACID ALLOWANCES FOR GROWING CHICKS INCLUDING BROILERS*

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*Synopsis*

Since amino acid requirements of chicks can be expressed accurately only for rather specific dietary nutrient combinations, environments, strains, and age, amino acid allowances for use in practical ration formulation have been developed. These amino acid allowances are not intended to be quantitatively higher than actual requirements, except perhaps for the potentially limiting amino acids which are subject to variation in availability and amount present in usual feedstuffs. These allowances are expressed as a function of metabolizable energy concentration of the diet for broiler chicks of similar environmental conditions and age.

Practical studies reveal that the Maryland minimum amino acid-energy ratios are adequate provided that the protein level is sufficiently high. Increasing the level of protein above that needed to supply all the essential amino acids results in a material increase in feed efficiency, with little if any, effect on weight gain. This is accompanied by a slight reduction in body fat content. Specific amino acid supplementation, or addition of glutamic acid to supply amino nitrogen, failed to produce the same response. The reduction in energy intake as a result of increasing the protein level is believed to be due to an effect on appetite. Increasing the protein level, with or without increasing the levels of the first limiting amino acids in isocaloric diets reduced relative energy intake per unit gain with no adverse effect on weight gain except at high ambient temperatures. Under hot weather conditions, the minimum amino acid allowances should be raised as feed intake is lowered, but good amino acid balance should also be maintained.

*Introduction*

NUTRITIONAL requirements of poultry have customarily been expressed in terms of concentration of nutrient per unit weight of ration. This method is not entirely accurate as the nutrient intake depends on the amount of diet consumed, which in turn may be influenced materially by several factors. Protein and amino acid intakes of chicks are especi-

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ally subject to the requirement that the daily intakes at any given stage of development must not fall below the necessary minima. Though energy concentration of the diet may largely determine diet consumption, activity and environmental conditions, as well as other dietary characteristics including protein level and quality, may also exert important effects. Changes in the relative protein and energy intakes also influence the body composition of the growing chicks, and there are differences in retention of dietary nitrogen as well (Combs, 1964*a*, Combs, Bossard, Childs & Blamberg, 1964). Since amino acid requirements of chicks can be expressed accurately only for rather specific nutrient combinations, environments, strains, and age, it has been necessary to work out empirical amino acid allowances for use in practical ration formulation until more exact information is available. The task is further complicated by normal variation in level and availability of the most limiting amino acids in feedstuffs.

Amino acid requirements of chicks are sometimes expressed in relation to the dietary protein or the energy content, as well as being stated as percentage of weight of diet. None of these methods is beyond criticism, as the apparent amino acid needs of the growing chick may be modified by:

1. factors which influence voluntary food intake and, in turn, the quantitative amount of each amino acid consumed;
2. differences in availability of the ingested amino acids; and
3. changes in metabolic efficiency with which the amino acids are utilized for growth and tissue repair.

Specific factors known to influence the voluntary feed consumption of growing chicks include dietary energy content, protein level, amino acid adequacy and balance, ambient temperature, activity, palatability and physical nature of the feed, growth rate and nutritional requirements of the chick.

To express the amino acid requirement as percentage of protein in the diet is sound when the diet contains just the 'optimal' level of balanced protein, but the percentage of an essential amino acid required in the protein generally becomes progressively lower as the level of protein in the diet is elevated above that needed to furnish adequate amounts of other amino acids. However, as the percentage of dietary protein is increased, the minimum levels of most essential amino acids also appear to be increased when they are expressed as percent of diet. Recent work suggests that this is due to an effect of protein level on voluntary food consumption (Combs, 1965).

Expression of requirements as a function of energy is still, in theory, not completely satisfactory since several factors remain to be considered. However, under most practical conditions this method probably is the one of choice. It works satisfactorily when the protein level and quality do not greatly differ for chicks of similar age and

growth rate maintained under similar environmental conditions. The method in effect embodies consideration of both the percentage amino acid required in 'effective protein' and the desired ratio of 'effective protein' to dietary energy. It takes into account the effect of dietary energy level in exerting a major effect on voluntary feed consumption under most conditions.

Availability of amino acids in a protein mixture obviously will influence the apparent dietary requirements for the amino acid. Certain ingredients appear to vary considerably with respect to availability of specific amino acids, including lysine and methionine, but considerably more work is required before rapid methods of estimating

TABLE 1

*Effect of energy intake on nitrogen retention of chicks*[1]
*University of Maryland*

| Grames N consumed (bird/day) | Metabolizable kcal consumed (bird/day) | 11-Day gain, (g.) | Percentage dietary N retained | Percentage carcass fat |
|---|---|---|---|---|
| 0·70 | 138 | 168 | 49·7 | 16·3 |
| 0·68 | 110 | 126 | 38·2 | 13·5 |
| 0·70 | 81 | 95 | 33·0 | 8·7 |
| 0·70 | 54 | 57 | 22·3 | 3·6 |

[1] White Rock males from 17th to 28th day of age. Protein composed of 48·32 parts corn, 35 parts dehulled soya bean meal, 0·05 parts lysine and 0·25 parts glycine.

differences in availability of the specific amino acids can be routinely used in quality control.

The efficiency of utilization of an amino acid inside the body is influenced by its level of intake in relation to the chick's need, to specific amino acid imbalances, and to the relative energy intake. With practical diets, broiler chicks will retain in their bodies from 50 to 55% of the dietary nitrogen. This would be approximately 60 to 65% retention of absorbed nitrogen, assuming 85% digestibility. Approximately one-half of the digestible nitrogen not retained can be attributed generally to certain amino acid surpluses even when minimal protein levels are used.

In earlier studies at Maryland, chicks were pair-fed so that protein and amino acid intakes were virtually identical but intakes of metabolizable energy were quite different. The results, given in Table 1, show the very striking effect of energy restriction on the retention of dietary nitrogen in the carcass, which drops from 50 to 22%. Body composition also was changed as shown by the differences in fat content. Either carcass fat or nitrogen content can be used as an index of the relative amount of energy available to spare protein metabolism. Chicks of the same age have essentially the same water/nitrogen ratio, but this decreases, from 25 to 16 : 1 with increasing age.

The effect of an amino acid deficiency is quite different from the effect of a low protein diet on relative voluntary energy consumption and the resulting body composition of chicks. Data are given in Table 2 which show the effect of graded levels of methionine without

TABLE 2

*Effect of methionine adequacy of isocaloric 20% protein diets on voluntary energy intake and body composition*[1]

| Average methionine (M) mg. M/kcal | Average daily weight gain (g.) | Methionine kcal/g. weight gain | Carcass analysis, per cent | | |
|---|---|---|---|---|---|
| | | | Moisture | Protein | Non-protein solids |
| | | *Average, 2 experiments (11-25th day)* | | | |
| ·425 | 6·2 | 10·5 | 67·1 | 18·9 | 14·0 |
| ·560 | 12·0 | 7·0 | 65·9 | 18·6 | 15·6 |
| ·695 | 18·6 | 5·8 | 65·3 | 18·3 | 16·4 |
| ·831 | 20·1 | 5·6 | 65·1 | 18·1 | 16·8 |
| ·966 | 20·6 | 5·4 | 65·3 | 18·2 | 16·5 |
| | | *Average, 3 experiments (0-21st day)* | | | |
| ·425 | 4·3 | 8·6 | 68·6 | 17·9 | 13·5 |
| ·492 | 6·7 | 7·3 | 67·2 | 17·9 | 14·9 |
| ·560 | 10·0 | 6·4 | 67·0 | 17·9 | 15·1 |
| ·628 | 12·7 | 5·9 | 66·9 | 17·6 | 15·5 |

[1] 4 replicas of 10 White Rock male chicks per treatment in each experiment.

change in the protein or nitrogen content of the total diet. In these trials and in others conducted with graded levels of lysine intakes, no increase in body fat content has been evident as the diet becomes progressively less adequate in respect of a single amino acid. In fact, the voluntary energy consumption of chicks fed diets having the same protein level but becoming increasingly deficient in a single amino acid, appears to decrease slightly in relation to their needs as indicated by body composition data.

### *Amino Acid Requirement Equation*

Perhaps the amino acid requirements can best be stated in terms of specific quantities of each amino acid needed for maintenance and for gain of tissue nitrogen or body weight of specified composition. Although this approach is considered sound, it lacks much in terms of ease of application.

An equation has been developed at Maryland (Combs, 1964*b*) for calculating total sulphur amino acid requirements of growing chicks in terms of maintenance and gain in body weight of a specific composition. The equation is:

$$R\ (c+m) = \frac{(C^2+1{\cdot}5C-5)\ W}{500} + (0{\cdot}65+3{\cdot}58)\ G$$

where

$R(c+m)$ = mg. of methionine + cystine required per chick per day,
$C$ = per cent nitrogen content of fasted carcass (moisture-free basis),
$W$ = average fasted body weight maintained during period, and
$G$ = average grams gain in body weight per chick per day.

Use of this equation to predict the requirements of growing broilers reared in floor pens and fed diets limiting or just adequate in total sulphur amino acids has been most encouraging. The predicted values averaged 96·8% (±4·2) of the calculated actual intakes in two tests with broilers reared to 4·5 weeks of age and fed a total of seven different diets. For broilers from 4·5 to 8 weeks of age, the predicted requirements in four different trials averaged 94·6%(±7·2) of the calculated intakes. These trials involved a total of 14 diet comparisons.

### *Amino Acid Allowances*

Even though the apparent amino acid requirements of chicks as usually expressed are subject to changes brought about by the several factors mentioned, it is desirable to use allowances as guides expedient for diet formulation. Since broiler chicks are of similar breeding, are reared under somewhat comparable environmental conditions at any specific age, and are not likely to be fed radically imbalanced diets containing excessive protein levels, it is reasonably satisfactory to express the amino acid allowances of the growing broiler as a function of the metabolizable energy content of the diet. Amino acid allowances need not be quantitatively higher than actual requirements, except that some consideration should be given to the likely variation of content and availability of those essential amino acids which may first be limiting in the diet.

In most practical diets containing corn and soya bean protein, lysine and total sulphur amino acids are the first two limiting amino acids, with threonine, glycine, tryptophan, isoleucine and valine becoming next limiting in no clearly predictable order. The other amino acids are generally supplied in adequate quantities by most practical protein mixtures and have received little experimental attention.

For these reasons, several studies have been conducted with male broilers reared in floor pens to determine the optimal levels of lysine and total sulphur amino acids. Summaries of the data pertaining to graded levels of methionine + cystine during the first 4½ weeks of age and from 4½-8 weeks of age are given in Tables 3 and 4 respectively. Similar data on lysine levels in starting rations were obtained in three trials and with finishers in four trials (Combs & Nicholson, 1962).

## TABLE 3

*Summary of six trials involving practical broiler finishing rations containing different levels of sulphur amino acids*

| % Protein | % ÷Mcal. ME/lb Lysine | Cystine | Meth. + cystine | Rel. gain in body wt., %[1] | Rel. energy uptake/unit wt., % | Mg. meth.+ cyst. cons/gm. gain |
|---|---|---|---|---|---|---|
| | *Exp. S-37—4 lots of 150; 4·5-8 wks. (1473 cal/lb) completed in May* | | | | | |
| 17·8 | ·719 | ·19 | ·421 | 97·0 | 104·8 | 14·14 |
| 17·8 | ·719 | ·19 | ·445 | 96·0 | 104·8 | 14·96 |
| 17·8 | ·719 | ·19 | ·470 | 97·5 | 104·1 | 15·71 |
| 21·8 | ·808 | ·21 | ·514 | 100 (1·99)[2] | 100 (7·18) | 16·50 |
| | *Exp. S-40—8 lots of 150: 4·5-8 wks (1470 cal/lb) completed in Feb.* | | | | | |
| 18·7 | ·71 | ·20 | ·401 | 95·7 | 104·5 | 15·70 |
| 18·7 | ·71 | ·20 | ·441 | 98·8 | 193·5 | 17·11 |
| 18·7 | ·71 | ·20 | ·481 | 100·8 | 100·2 | 18·05 |
| 18·7 | ·71 | ·20 | ·520 | 100 (1·67) | 100 (8·27) | 19·49 |
| | *Exp. 41 CP—7 lots of 50; 4·5-7·5 wks (1439 cal/lb) completed in Feb.* | | | | | |
| 19·0 | ·74 | ·21 | ·416 | 97·8 | 101·2 | 13·78 |
| 19·0 | ·74 | ·21 | ·457 | 96·9 | 101·0 | 15·13 |
| 19·0 | ·74 | ·21 | ·499 | 99·7 | 99·1 | 16·16 |
| 19·0 | ·74 | ·21 | ·540 | 100 (1·81) | 100 (7·20) | 17·64 |
| | *Exp. S-41[3]—4 lots of 150; 4·5-8 wks (1534 and 1524 cal/lb) completed in June* | | | | | |
| 19·2 | ·74 | ·194 | ·389 | 97·9 | 103·4 | 14·33 |
| 19·2 | ·74 | ·194 | ·430 | 93·3 | 104·3 | 15·97 |
| 19·2 | ·74 | ·194 | ·471 | 101·0 | 99·1 | 16·61 |
| 19·2 | ·74 | ·194 | ·512 | 100 (1·94) | 100 (7·85) | 18·21 |
| 20·7 | ·75 | ·219 | ·417 | 97·9 | 103·8 | 15·52 |
| 20·7 | ·75 | ·219 | ·450 | 99·0 | 99·6 | 16·05 |
| 20·7 | ·75 | ·219 | ·483 | 99·0 | 101·7 | 17·59 |
| 20·7 | ·75 | ·219 | ·516 | 100 (1·91) | 100 (7·90) | 18·47 |
| | *Exp. S-42[3]—4 lots of 150; 4·5-8 wks (1534-1524 cal/lb) completed in Sept.* | | | | | |
| 19·2 | ·74 | ·194 | ·389 | 94·5 | 104·6 | 13·55 |
| 19·2 | ·74 | ·194 | ·430 | 96·3 | 104·1 | 14·92 |
| 19·2 | ·74 | ·194 | ·471 | 97·1 | 99·4 | 15·60 |
| 19·2 | ·74 | ·194 | ·512 | 100 (1·82) | 100 (7·34) | 17·03 |
| 20·7 | ·75 | ·219 | ·417 | 98·7 | 104·1 | 13·49 |
| 20·7 | ·75 | ·219 | ·450 | 96·3 | 105·0 | 15·30 |
| 20·7 | ·75 | ·219 | ·483 | 97·3 | 101·4 | 15·82 |
| 20·7 | ·75 | ·219 | ·516 | 100 (1·86) | 100 (7·12) | 16·66 |
| | *Exp. S-43—8 lots of 150; 4·5-7·5 wks (1534, 1529 cal/lb) completed in Dec.* | | | | | |
| 19·2 | ·74 | ·194 | ·389 | 95·3 | 106·4 | 13·73 |
| 19·2 | ·74 | ·194 | ·442 | 98·1 | 104·8 | 15·53 |
| 19·2 | ·74 | ·194 | ·495 | 100·9 | 101·5 | 16·85 |
| 23·1 | ·88 | ·233 | ·498 | 100 (1·57) | 100 (7·38) | 16·67 |

[1] Expressed as relative growth and metabolizable energy intake per unit body weight gain as % of control ration within each trial.

[2] Values in parentheses refer to average pounds body weight gain or metabolizable kilocalories consumed per gram weight gain for the control reference group.

[3] The 20·7% protein diets in these series contained 2% hydrolised feather meal.

## TABLE 4

*Results obtained with male White Rock broiler chicks kept in floor pens and fed starting rations containing different levels of methionine and cystine (University of Maryland Trials)*

| Protein/M. E. per lb. | % ÷ Mcal. M. E.[1] Lysine | Cystine | Methionine + cystine | Body wt. rel. %[2] | Energy/unit wt., rel. %[2] |
|---|---|---|---|---|---|
| | | *Exp. 1-6 lots of 40, 0-3·5 wks* | | | |
| 21·8/1513 | 0·79 | 0·215 | 0·430 | 88·1 | 111·4 |
| | | | 0·471 | 97·3 | 105·1 |
| 25·1/1478 | 0·97 | 0·249 | 0·545 | *100* | *100* |
| | | *Exp. 2—2 lots of 30, 0-4·5 wks* | | | |
| 20·7/1368 | 0·80 | 0·234 | 0·469 | 94·2 | 108·2 |
| | | | 0·493 | 98·1 | 102·4 |
| | | | 0·542 | 103·2 | 101·8 |
| 22·8/1346 | 0·93 | 0·260 | 0·555 | 100 | 100 |
| | | *Exp. 2a—2 lots of 50, 0-4 wks* | | | |
| 22·8/1528 | 0·85 | 0·226 | 0·448 | 84·1 | 106·7 |
| | | | 0·493 | 94·8 | 104·3 |
| | | | 0·539 | 97·8 | 98·8 |
| 25·7/1502 | 1·01 | 0·257 | 0·554 | *100* | *100* |
| | | *Exp. 3—3 lots of 40, 0-4 wks* | | | |
| 20·8/1421 | 0·78 | 0·228 | 0·457 | 89·4 | 107·1 |
| | | | 0·496 | 91·7 | 100 |
| | | | *0·535* | *96·9* | *106·1* |
| 24·0/1397 | 0·91 | 0·255 | 0·548 | 100 | 100 |
| | | *Exp. 3a—3 lots of 50, 0-4 wks* | | | |
| 20·8/1421 | 0·78 | 0·228 | 0·457 | 90·6 | 109·1 |
| | | | 0·496 | 93·9 | 105·6 |
| 24·0/1397 | 0·91 | 0·255 | 0·548 | 100 | 100 |
| | | *Exp. 4—2 lots of 50, 0-4 wks* | | | |
| 23·1/1539 | 0·85 | 0·222 | 0·446 | 91·4 | 107·8 |
| | | | 0·491 | 98·4 | 105·2 |
| | | | 0·514 | 100 | 99·3 |
| | | | 0·537 | 100 | 100 |
| | | *Exp. 5—2 lots of 50, 0-4 wks*[3] | | | |
| 21·9/1450 | 0·83 | 0·233 | 0·466 | 90·7 | 104·3 |
| | | | *0·514* | *98·4* | *103·1* |
| | | | 0·534 | 94·6 | 106·8 |
| | | | 0·554 | 100 | *100* |
| | | *Exp. 6—2 lots of 50, 0-4 wks.*[3] | | | |
| 21·9/1450 | 0·83 | 0·233 | 0·466 | 93·3 | 113 9 |
| | | | 0·490 | *97·0* | *104·9* |
| | | | 0·514 | 100 | 101 2 |
| | | | 0·534 | *98·5* | *103 1* |
| | | | 0·554 | *100* | *100* |

[1] Calculated from University of Maryland *1962 Table of Ingredient Composition*

[2] Expressed as rel. growth and metab. energy intake per unit body wt. as a rel % of adequate control ration within each experiment.

[3] In Exps. 5 and 6, lysine additions failed to improve results when added in addition to an adequate amount of meth.+cystine. Also, cystine additions alone improved growth and feed efficiency.

From these data, regression equations were developed (Combs, 1963, 1964*b*) for lysine and for total sulphur amino acid (in absence of excess cystine) requirements of broilers for body weight gain and for relative energy uptake per unit gain (Fig. 1). Each of these was related to the log of the amino acid-energy ratio (expressed as percentage amino acid in ration divided by metabolizable megacalories per pound). These regression equations are listed opposite.

From these equations it is possible to estimate the approximate change in relative body weight gain and relative energy uptake per unit gain which can be expected if the levels of these amino acids are progressively reduced from the levels shown to be optimal by these equations (Table 5). From the values it is clear that the response to both lysine and total sulphur amino acids is more striking during the starting period than during the finishing period.

The levels of all essential amino acids recommended for use in broiler rations, expressed as amino acid energy ratios are given in

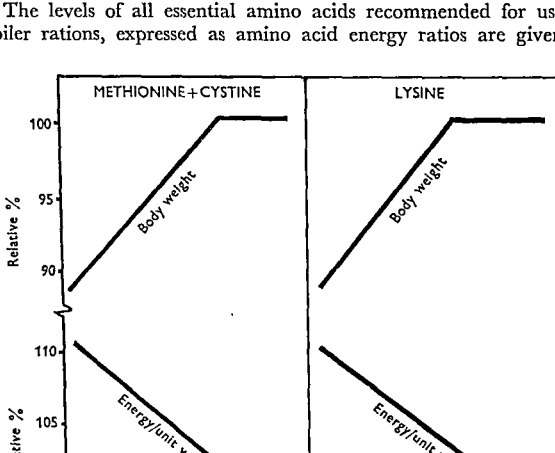


FIG. 1. Response of male broilers to different lysine and sulphur amino acid levels during starting period expressed as a function of metabolizable energy content of the ration. By plotting $x$, log (% amino acid ÷ metab. calories per pound) and $y$, % relative weight gain (above) or % relative energy intake per unit gain (below), the minimal requirement for the limit of response is at the point where the response plateaus.

| Ration | Amino Acid | Regression equation*† |
|---|---|---|
| | (For relative body weight gain ($\hat{y}$) | |
| Starting | Methionine+cystine | $\hat{y}=131{\cdot}28+117{\cdot}4 \log x$ |
| Starting | Lysine | $\hat{y}=115{\cdot}9+168{\cdot}2 \log x$ |
| Finishing | Methionine+cystine | $\hat{y}=109{\cdot}94+34{\cdot}75 \log x$ |
| Finishing | Lysine | $\hat{y}=113{\cdot}6+92{\cdot}14 \log x$ |
| | (For relative energy uptake ($\hat{y}$)) | |
| Starting | Methionine+cystine | $\hat{y}=77{\cdot}59-88{\cdot}59 \log x$ |
| Starting | Lysine | $\hat{y}=94{\cdot}7-91{\cdot}58 \log x$ |
| Finishing | Methionine+cystine | $\hat{y}=87{\cdot}15-44{\cdot}05 \log x$ |
| Finishing | Lysine | $\hat{y}=87{\cdot}58-83{\cdot}46 \log x$ |

* Where $x$ is percentage of the amino acid in the ration divided by the metabolizable megacalories per lb.

† $\hat{y}$=% relative gain or energy uptake.

Table 6. These values for amino acids other than methionine, cystine, lysine, arginine, and threonine are based to a large extent on requirements obtained from the National Research Council. The threonine requirement is based on the work of Anderson (1961) and Dobson, Anderson and Warmick (1964) at Utah State University. Table 6 also

TABLE 5

*Effect of reduction of amino acid levels on weight gains and energy uptake by broilers based on regression equations*[1]

| | Methionine+Cystine | | Lysine | |
|---|---|---|---|---|
| Relative gain, % | Starters | Finishers | Starters | Finishers |
| | % Amino acid/Mcal M.E./lb | | | |
| 100 | ·541 | ·517 | ·804 | ·711 |
| 99 | ·526 | ·484 | ·793 | ·694 |
| 98 | ·516 | ·453 | ·783 | ·677 |
| 97 | ·507 | ·424 | ·772 | ·660 |
| 96 | ·497 | ·397 | ·761 | ·644 |
| 95 | ·480 | ·372 | ·751 | ·628 |
| % Relative energy uptake | % Amino acid/Mcal M.E./lb | | | |
| 100 | ·559 | ·511 | ·875 | ·710 |
| 101 | ·544 | ·485 | ·853 | ·691 |
| 102 | ·530 | ·459 | ·832 | ·672 |
| 103 | ·517 | ·437 | ·812 | ·654 |
| 104 | ·503 | ·414 | ·792 | ·636 |
| 105 | ·491 | ·393 | ·772 | ·618 |

[1] Based on regression equations given in text.

includes desirable energy-protein ratios which should be met even though adequate levels of essential amino acids may be supplied with less total protein.

A comparison of these 'amino acid-energy ratios' recommended allowances with those calculated from requirement values for starting

chicks as given by the United States National Research Council (NRC), the British Agricultural Research Council (ARC), the amino acid reference diet of Dean and Scott (1965), and the balanced protein mixture of Anderson (1961) is given in Table 7. Note that the Maryland values are lower than NRC values for arginine, methionine, and total sulphur amino acids, but higher for lysine and threonine. Although the values of Dean and Scott (1965) obtained with amino acid

TABLE 6

*Recommended amino acid-energy ratios for broiler rations by periods (University of Maryland)*

| | Per cent A. A. ÷ Meal M.E./lb | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0-2·5 wks | | 2·5-5 wks | | 5-8 wks | | After 8 wks | |
| Amino Acid | Winter | Summer | Winter[1] | Summer | Winter[2] | Summer | Winter | Summer |
| Methionine | ·31 | ·31 | ·28 | ·29 | ·25 | ·27 | ·21 | ·24 |
| Methionine+ cystine | ·62 | ·62 | ·56 | ·59 | ·51 | ·56 | ·43 | ·48 |
| Lysine | ·96 | ·96 | ·87 | ·91 | ·71 | ·78 | ·60 | ·67 |
| Tryptophane | ·18 | ·18 | ·16 | ·17 | ·13 | ·14 | ·11 | ·12 |
| Threonin | ·63 | ·63 | ·57 | ·60 | ·49 | ·53 | ·42 | ·46 |
| Arginine | ·88 | ·88 | ·80 | ·84 | ·70 | ·77 | ·60 | ·66 |
| Glycine | ·83 | ·83 | ·75 | ·79 | ·64 | ·70 | ·54 | ·61 |
| Phenylalanine | ·60 | ·60 | ·55 | ·58 | ·47 | ·52 | ·40 | ·45 |
| Phenylalanine+ tyrosine | 1·20 | 1·20 | 1·09 | 1·14 | ·94 | 1·03 | ·80 | ·89 |
| Valine | ·68 | ·68 | ·62 | ·65 | ·52 | ·57 | ·44 | ·49 |
| Isoleucine | ·51 | ·51 | ·46 | ·48 | ·41 | ·45 | ·35 | ·39 |
| Leucine | 1·20 | 1·20 | 1·09 | 1·14 | ·94 | 1·03 | ·80 | ·89 |
| Histidine | ·26 | ·26 | ·24 | ·25 | ·21 | ·23 | ·18 | ·20 |
| Desirable C/P ratio* | 60 | 60 | 66 | 63 | 75 | 69 | 88 | 79 |

[1] Present University of Maryland minimum values for broiler starters (winter) where no pre-starter feed is used.

[2] Present University of Maryland minimum values for broiler finisher feeds where no special terminal feed is used.

* Metabolizable energy values.

diets are lower throughout due to differences in digestibility and differences in total relative energy intake of chicks fed such diets. The agreement is very close to the NRC values when comparisons are based on proportions of one amino acid to another. Arginine, methionine, methionine+cystine, phenylalanine, phenylalanine+tyrosine and leucine are relatively lower than the NRC requirements, and the proportions of lysine, threonine, and isoleucine are higher. The ARC values for tryptophan, threonine, and isoleucine would appear to be low.

### *Practical Studies*

In floor pen studies designed to test these minimum (winter) values in starting feeds to 4·5 or 5 weeks, a series of rations was fed with increas-

## TABLE 7

*Comparison of recommended amino acid-energy ratios (starting chicks)*

| | NRC[1] Requirements | ARC[2] Requirements | Univ. Illinois[3] Amino acid reference diet | Univ. Utah[4] balanced mixture | Univ. Maryland[5] minimum allowances |
|---|---|---|---|---|---|
| Metabolizable kcal/gm | 2·86 | 2·8 | 4·1 | 3·15 | 3·2 |
| Arginine | 4·20 | — | 2·68 | 4·50 | 3·63 |
| Lysine | 3·50 | 3·57 | 2·74 | 4·22 | 3·94 |
| Histidine | 1·05 | 1·25 | 0·73 | 1·49 | 1·09 |
| Methionine | 1·57 | 1·00 | 1·10 | — | 1·27 |
| Meth.+cystine | 2·80 | 2·50 | 1·95 | 2·54 | 2·54 |
| Tryptophan | 0·70 | 0·54 | 0·55 | 0·70 | 0·73 |
| Phenylalanine | 2·45 | 2·14 | 1·66 | — | 2·50 |
| Phe.+tyr. | 4·90 | 4·28 | 3·20 | 4·73 | 4·95 |
| Leucine | 4·90 | 5·36 | 2·93 | 4·85 | 4·95 |
| Isoleucine | 2·10 | 1·43 | 1·95 | 2·82 | 2·09 |
| Threonine | 2·10 | 1·57 | 1·59 | 2·70 | 2·60 |
| Valine | 2·80 | 2·85 | 2·00 | 3·17 | 2·81 |
| Glycine | 3·50 | 3·57 | 3·90* | — | 3·40 |
| Proline | — | — | 2·44* | — | — |
| Glutamic acid | — | — | 29·3* | — | — |
| Protein | 70·0 | — | 43·2 | 63·5 | 69·0 |

[1] National Research Council, National Academy of Sciences (1960).
[2] Agricultural Research Council, London, 1963.
[3] Dean and Scott (1965).
[4] Anderson (1961).
[5] Combs (1965).
* Not minimal levels.

## TABLE 8

*Results obtained in Maryland broiler trials S-48 and S-49 with separated broiler males and females with diets varying in protein without change in amino acid quality (starting period)*

| % Dietary protein | Relative % amino acid adeq. | Average weight, 4½ wks.[1] Males | Females | Average | Feed cons./weight (0-4½ wks.)[1] Males | Females | Average |
|---|---|---|---|---|---|---|---|
| | | | *Trial S-48* | | | | |
| 20·3 | 88 | 1·38 | 1·26 | 1·32 | 1·69 | 1·74 | 1·72 |
| 22·9 | 100 | 1·48 | 1·33 | 1·40 | 1·59 | 1·61 | 1·61 |
| 25·5 | 112 | 1·53 | 1·36 | 1·45 | 1·52 | 1·56 | 1·54 |
| 28·1 | 124 | 1·60 | 1·41 | 1·51 | 1·45 | 1·50 | 1·48 |
| | | | *Trial S-49* | | | | |
| 22·9 | 100 | 1·72 | 1·46 | 1·59 | 1·50 | 1·60 | 1·55 |
| 25·5 | 112 | 1·73 | 1·52 | 1·62 | 1·46 | 1·52 | 1·46 |
| 28·1 | 124 | 1·72 | 1·50 | 1·61 | 1·42 | 1·51 | 1·47 |
| 30·7 | 136 | 1·73 | 1·50 | 1·61 | 1·43 | 1·48 | 1·46 |

[1] Each value based on four groups of 150 broilers each

ing levels of protein and correspondinng increases in the levels of all essential amino acids. The results of these trials (Combs & Nicholson, 1962) showed that better feed conversion was obtained without appreciable difference in growth. Runnels (1964) has summarized the results

TABLE 9

*Effect of protein and amino acid levels on gain and feed efficiency of broilers during the starting period*
*Poultry department, Univ. of Maryland*[1]

| % Protein | MC/P ratio | % Relative adequacy, 1st limiting amino acid | Body weight, gm | % Relative energy uptake/gain |
|---|---|---|---|---|
| | | *Trial CP-44 (35 days)* | | |
| 20·8 | 71·5 | 100 | 817 | 100 |
| 23·0 | 65 | 110 | 849 | 96·6 |
| 25·3 | 59·4 | 120 | 849 | 93·5 |
| 27·5 | 53·2 | 130 | 849 | 93·7 |
| 23·0 | 65 | 100 | 844 | 96·5 |
| 25·3 | 59·4 | 100 | 844 | 94·8 |
| | | *Trial S-45 (32 days)* | | |
| 22·2 | 65·3 | 100 | 622 | 100 |
| 24·0 | 63·5 | 110 | 636 | 97·8 |
| 26·2 | 55·3 | 120 | 654 | 94 |
| 24·0 | 60·4 | 100 | 649 | 95 |
| 26·0 | 55·8 | 100 | 663 | 92·5 |
| | | *Trial CP-45 (24 days)* | | |
| 22·2 | 65·3 | 100 | 422 | 100 |
| 24·2 | 59·9 | 110 | 472 | 95 |
| 26·2 | 55·3 | 120 | 499 | 92·3 |
| 24·0 | 60·4 | 100 | 463 | 93·4 |
| 26·0 | 55·8 | 100 | 481 | 92·8 |
| | | *Trial S-47 (32 days)* | | |
| 22·5 | 65·2 | 100 | 804 | 100 |
| 24·4 | 60·1 | 106 | 785 | 98·8 |
| 25·3 | 57·9 | 112 | 804 | 96·3 |
| 28·1 | 52·2 | 124 | 844 | 91·7 |
| 25·5 | 57·5 | 100 | 795 | 97·8 |
| 25·5 | 57·5 | 100 | 831 | 95·7 |
| | | *Trial BCF (7-26 days gain)* | | |
| 22·5 | 65·2 | 100 | 421 | 100 |
| 25·3 | 57·9 | 112·5 | 438 | 96·2 |
| 28·1 | 52·2 | 125 | 432 | 91·8 |
| 33·7 | 43·5 | 150 | 442 | 88·6 |
| 39·4 | 37·2 | 175 | 418 | 89·2 |
| 45·0 | 32·6 | 200 | 404 | 87·3 |

of several commercial feeding trials involving linear programmed formulas varying from 95 to 125% of the earlier Maryland minimum amino acid-energy values in both starter and finisher feeds, with corresponding changes in protein level. His data suggests the need

for increasing the present Maryland minima by approximately 5-10% for starters (winter, 2·5-5 weeks) and up 5% for the 5-8 week (winter) minimums for finishers as given in Table 7.

Recent studies in Maryland, in which protein and amino acid levels were increased in balance from 90 to 136% of the Maryland minimum estimates, are summarized in Table 8. Both males and females showed improved feed efficiency with isocaloric starting diets (1466 kcal per lb) in these tests throughout the levels studied.

In view of the effect of protein level *per se* on voluntary food intake discussed previously, a number of studies have been conducted with broilers to determine the effect of increasing the protein level without

TABLE 10

*Effect of protein and amino acid levels on gain and feed efficiency of finishing broilers (4·5-8 weeks) Univ. of Maryland*[1]

| % Protein | MC/P ratio | % Relative adequacy, 1st limiting amino acid | Weight gain, lb | Feed/gain | % Relative energy uptake |
|---|---|---|---|---|---|
| | | *Trial S-44 (completed in March)* | | | |
| 18·2 | 82·9 | 90 | 1·54 | 2·69 | 106 |
| 19·7 | 76·6 | 100 | 1·57 | 2·54 | 100 |
| 21·9 | 68·9 | 110 | 1·60 | 2·45 | 96·5 |
| | | *Trial S-46 (completed in July)* | | | |
| 19·8 | 78·2 | 90 | 1·74 | 2·33 | 101 |
| 19·8 | 78·2 | 100 | 1·73 | 2·31 | 100 |
| 21·7 | 70·4 | 100 | 1·81 | 2·26 | 96·7 |
| 23·7 | 64·5 | 100 | 1·78 | 2·29 | 98 |
| | | *Trial S-47 (completed in October)* | | | |
| 19·8 | 78·2 | 90 | 1·92 | 2·38 | 102 |
| 19·8 | 78·2 | 100 | 2·00 | 2·33 | 100 |
| 21·7 | 70·4 | 100 | 2·05 | 2·28 | 96·2 |
| 23·7 | 64·5 | 100 | 2·08 | 2·24 | 94·7 |

raising the levels of the first limiting amino acids (methionine+cystine and lysine) as compared with raising the amino acid levels and protein in balance. The data from five such trials are given in Table 9 for starting feeds and in Table 10 for finishers. These results show that in both starter and finisher feeds, the voluntary energy uptake per unit gain is decreased as the level of protein is raised above that needed to supply the minimum levels of essential amino acids, based on our estimates.

In these trials, the improvement in feed efficiency has been as great when the protein level is raised without increasing the levels of the first limiting amino acids as when all amino acid levels were increased in balance. The results of four starting trials, in which the protein levels

were raised in balance and not in balance, are shown in Fig. 2. This reduced energy uptake has been accompanied by slightly lower carcass fat content.

The Maryland minimum estimates for lysine and total sulphur amino acids (TSAA) appear to be sound, but the addition of more protein than is necessary to meet these requirements improves feed efficiency. In fact, the actual intakes of lysine and methionine+cystine in several trials are decreased as the protein level is increased and better efficiencies obtained.

This reduction in energy intake from additional protein is believed

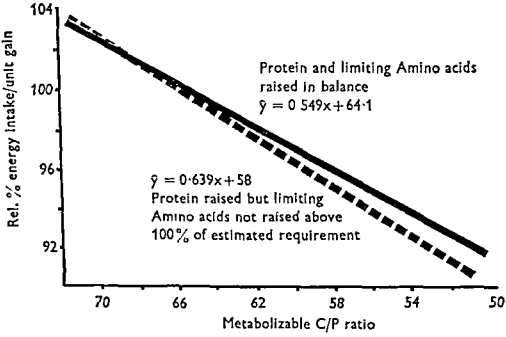


FIG. 2. Effect of calorie/protein ratio on energy efficiency and feed conversion (summary of trials CP-44, S-45, CP-46, and S-47).

to be due to an effect on appetite. It is possible, of course, that the level of some amino acid, other than lysine or TSAA, is suboptimal and that this is increased as the protein level is raised. To study this possibility, amino acids were added in two of these trials. A slight improvement was obtained from the addition of lysine and methionine alone, indicating that one or both were marginal as calculated. The further addition of glycine did not materially improve the results over lysine and methionine alone. The addition of different amino acid mixtures had no further effect in trial CP-45. Studies designed to test the need for amino nitrogen have failed to show responses to glutamic acid. This effect may be explained in terms of a general effect on appetite rather than due to the addition of a limiting amino acid. Furthermore, calculated intakes of each essential amino acid do not support the specific limiting amino acid theory.

Similar improvements in feed efficiency (reduced energy uptake per unit gain) were obtained by adding hydrolysed feather meal, corn gluten meal, soya bean meal or soya bean meal and fish meal as a

means of increasing the protein level above that required to supply the calculated level of essential amino acids. Increasing the protein level during warm weather, however, was of no value unless the amino acid balance was maintained. This is as one might predict if the effect on appetite is through an increase in heat production during metabolism.

*Discussion and Conclusions*

Diets which have been 'imbalanced' by the addition of one or several (but not all) amino acids also depress feed consumption of chicks. Anderson, Combs, Groschke, and Briggs (1951) observed marked decreases in feed intake and growth of chicks from the addition of 4% of certain amino acids to the diet while the same level of others had little or no effect.

Similar reductions in ad libitum feed intake and growth retardation of chicks have been reported from the addition of a mixture of all essential amino acids except for the one first limiting. This has been demonstrated in diets first limiting in tryptophan, lysine or methionine by Fisher and Shapiro (1961) and in diets low in arginine, isoleucine, leucine, lysine and valine by Hill and Olson (1963).

We regularly use the terms 'balanced' and 'imbalanced' with respect to protein quality. There is some evidence suggesting specific interrelationships between lysine and arginine, and between leucine, isoleucine and valine, as well as the well-established relationship of methionine and cystine and that of phenylalanine and tyrosine. But there is considerable evidence to suggest that the effects of most imbalances are non-specific and that these function by influencing the physiological appetite and, in turn, feed intake.

Fisher and Shapiro (1961) have suggested that an amino acid imbalance exerts its effect by depressing voluntary food intake and actually creating a deficiency of the first limiting amino acid. They were able to change the feed intake of chicks by modification of the energy density of diets limiting in tryptophan, lysine or methionine. They found that 'imbalancing' amino acids had no apparent effect when the amount of the first limiting amino acid ingested was the same. Their studies included the measurement of nitrogen retention and body composition and revealed no differences in metabolic efficiency of the limiting amino acid; hence, the effect of the 'imbalance' appeared to be due to the depression in voluntary food consumption.

In short-term studies designed to measure feed consumption of chicks by hourly intervals, our group has shown that chicks which have been fasted for 12 hours will eat about the same amount of food during the first hour after fasting regardless of the adequacy of the amino acid balance. However, during subsequent hourly periods, groups of chicks fed markedly imbalanced or deficient diets eat very little as compared to controls fed balanced or complete diets.

This depression in *ad libitum* consumption after the first hour has been produced with protein mixtures deficient in a single amino acid as well as with diets containing an excess of a single or several amino acids. Since initial feed consumption is unaffected, this observation indicates that the desire to eat is reduced in some way due to the accumulation and removal of certain amino acids and their metabolites in the blood. If the protein level of the diet is not excessive and the amino acids in the protein are in good balance to meet the needs of the chick, one would expect rapid dissipation of the amino acids from the blood as protein is built in the body. If one or more is notably deficient in the relation to the levels of the others, then those present in higher amounts than can be used to form tissue proteins would remain until they are metabolized. The chick tends to eat brief meals at rather regular intervals. It is believed that the chick's desire to eat its next 'meal' is reduced when excesses of imbalanced amino acid mixtures are present in the blood. Studies with chicks and rats reveal imbalanced blood levels when such diets are fed.

Just how imbalanced amino acid blood levels exert an effect on appetite is not known. Studies at Maryland have revealed no meaningful differences in the blood glucose level, a quantity which has been associated with appetite and certain dietary variables. The non-protein nitrogen level of the blood is elevated as one might predict when imbalanced protein mixtures are fed. It is of interest to note that Almquist (1954) suggested that when amino acid deficient diets are fed, other amino acids would accumulate in the blood with a probable impairment of appetite.

One possible way in which increased plasma levels might reduce appetite is through increased amounts of free energy produced from the metabolism of amino acids. Brobeck (1948, 1960) has stressed the close connexion between body temperature and food intake and suggested that heat acts on sensitive neurons of the rostral hypothalamus and the preoptic area or the brain or directly upon neurons of the 'appetite centre'.

Andersson and Larsson (1961) were able to induce eating in the fed goat by local cooling of the preoptic and rostral hypothalamus, even at body temperatures above 41°C. Warming the same area inhibited eating in the hungry animal and induced drinking. Following inactivation of the preoptic 'heat loss centre' by proton irradiation, the goat continued to eat with a seemingly good appetite at body temperatures above 41°C, indicating that the effect of warming the preoptic area on appetite was not due to a direct thermal effect on the 'appetite centre'.

In this connection, Klain, Vaughn and Vaughn (1962) and Klain and Winders (1964) found that exposure of rats to cold (7°C) permitted them to increase food intake and overcome the growth depressing effects of amino acid imbalanced diets. Recent data from Maryland (1965), Kahlil, Combs, Blamberg and Vasaitis, show that growing

chicks fed a methionine-low diet gain more rapidly at temperatures of 52°F than at an ambient temperature of approximately 80°F. Also, a lower level of added methionine was needed to produce maximum growth in the cold environment due to the marked increase in total food intake. Blood serum amino acid analyses revealed only slight changes in levels except for methionine. Excess methionine levels, however, were more toxic in the cold environment, suggesting that the animal's ability to metabolize methionine was limited.

The studies described here reveal that increasing the protein level without change in quality results in reduced feed consumption per unit gain on isocaloric diets. This is true for males and females during the starting and finishing periods even though body weight is not affected. This can be expected even if the critically limiting amino acids are not raised above the Maryland minimum estimates, except under conditions of high ambient temperatures. Body fat content is lowered slightly as a result of the reduced energy intake caused by the excess protein in the diet.

The Maryland minimum estimates for amino acids are considered to be adequate provided that the MC/P ratios indicated in Table 6 are met. Increasing the protein level, in or out of balance, appears to reduce energy intake and improve feed efficiency, except under hot weather conditions. The magnitude of the effect is small so the potential importance of this relationship is entirely dependent on relative cost of protein and energy. Such an approach permits the use of higher levels of poor quality protein to supply the essential amino acids and this may be economically sound at certain prices. Under hot weather conditions, the minimum amino acid levels, expressed as percent of diet, should be increased to correct for reduced feed intake, but the best practical protein quality should be maintained.

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# 9

# AMINO ACID ALLOWANCES FOR LAYING HENS

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*Synopsis*

A description is given of an experimental programme in which 600 laying birds were fed diets of 10·5, 12·5 or 14·5% protein to which various amino acid mixtures had been added. The results indicate that the diet of 14·5% protein plus 0·025% tryptophan supported the best level of production. It is suggested that the amino acid levels in this diet are adequate to meet the amino acid requirements under these conditions. The concept of amino acid allowances is expressed in the form of intake of amino acid per unit time (mg amino acid per 1·8 kg bird per day). The current results are compared with other published values and the significance of substantial differences is discussed.

*Introduction*

It is recognized that the amino acid content of the diet is in practice of more importance than the total level of protein. It is, however, necessary to consider not only the levels of each individual amino acid but also the ratios between individual amino acids and the relative level of the non-essential amino acid component.

Many of the early studies to determine the amino acid requirements of the laying hen did not take full heed of the importance of the balance of amino acids. This probably contributed to some of the discrepancies in published values. In general, a single amino acid was considered in isolation. Thus Cravens (1948) studied isoleucine only, Leong and McGinnis (1952) methionine only, Machlin (1955) leucine only, Adkins, Miller, Bird, Elvehjem and Sunde (1958) threonine only and Adkins, Harper and Sunde (1962) arginine only. The general procedure adopted was that the test amino acid was added in graded amounts to a basal diet designed to be specifically deficient in that particular amino acid. The level of amino acid supporting the best level of production was deemed to represent the requirement. However, if the amino acid balance is not ideal and other amino acids are also present at less than the ideal level, the response to supplements of the first limiting amino acid will be halted at a barrier defined by the second most limiting amino acid.

Several attempts have been made to define amino acid allowances for laying hens using whole egg protein as a reference standard. Thus, Combs (1960, 1961) produced a formula for calculating the methionine requirement of the laying hen from a consideration of the factors which could affect the requirement. The allowances for the other amino acids were calculated from their ratio to methionine in whole egg protein. These values were found to be quite successful over a range of dietary 'energy' concentrations. Johnson and Fisher (1958) defined the allowance for lysine by the use of synthetic diets containing free amino acids. The recommended levels of the other amino acids were again calculated from their ratio to lysine in whole egg protein. Diets formulated upon such amino acid standards raised by 10% gave a satisfactory egg production but could not maintain egg weight. This

TABLE 1

*Composition of basal diets* (%)

| | | | |
|---|---|---|---|
| Protein (% N×6·25) | 10·5 | 12·5 | 14·5 |
| Maize meal | 50 | 45 | 35 |
| Ground wheat | 35 | 34 | 37·5 |
| White fish meal (65% protein) | 2·5 | 2·5 | 2·5 |
| Soya bean meal (50% protein) | 0 | 5 | 10 |
| Fat (HEF) | 2·5 | 3·5 | 5 |
| Limestone | 5 | 5 | 5 |
| Mineral and vitamin supplement | 5 | 5 | 5 |

was found to be due to a shortage of non-essential nitrogen, thus demonstrating the importance of a balance between essential and non-essential amino acids as well as between the essential amino acids themselves.

In some investigations a more direct approach has been adopted in that dietary amino acid levels are related to performance under specified conditions. In a preliminary trial (Taylor, Payne & Lewis, 1966) basal diets of 10·5% protein, 12·5% protein and 14·5% protein were prepared using wheat, maize and soya bean meal mixtures (Table 1). Free amino acids were added to these singly or in combination to produce 16 experimental diets devised to establish a sequential addition of certain potentially limiting amino acids. The results indicated that the 14·5% protein diet with a supplement of 0·025% tryptophan supported the best level of production. A diet of 12·5% protein plus 0·05% lysine, 0·025% tryptophan and 0·05% isoleucine also resulted in a good level of production. These two diets were selected as the basis for the next experimental programme (Table 2).

*Experimental*

Diet 1 contains 12·5% protein (N×6·25) plus 0·05% lysine, 0·025% tryptophan and 0·05% isoleucine, whilst diet 2 contains an additional 0·025% tryptophan, since this was thought to be the limiting amino acid. Diets 3 and 4 follow the amino acid patterns of diets 1 and 2,

respectively, with the threonine level reduced to that recommended by the National Research Council (1960). This was accomplished by adding essential amino acids other than threonine to the 10·5% protein diet. It was thought that the relatively high level of threonine in diets 1 and 2 could have been interacting adversely with the low level of tryptophan. Diet 5 is the 14·5% protein basal diet whilst diets 6, 7 and 8 are prepared from diet 5 by adding 0·0125%, 0·025% and ·00375% tryptophan respectively. These treatments were included to establish an appropriate allowance for tryptophan, since it had been shown in the preliminary trial that tryptophan was the limiting amino acid. Diets 9, 10, 11 and 12 are based upon diet 7 with additions of

TABLE 2

*Experimental treatments*
*(Lysine as L lysine monohydrochloride, all other amino acids as DL form)*

1. 12·5% protein diet+0·05% lysine+0·025% tryptophan+0·05% isoleucine
2. 12·5% protein diet+0·05% lysine+0·05% tryptophan+0·05% isoleucine
3. As 1 with threonine reduced (built up from 10·5% protein)
4. As 2 with threonine reduced (built up from 10·5% protein)
5. 14·5% protein basal diet
6. 14·5% protein basal diet+0·0125% tryptophan
7. 14·5% protein basal diet+0·025% tryptophan
8. 14·5% protein basal diet+0·0375% tryptophan
9. 14·5% protein basal diet+0·025% tryptophan+0·05% lysine
10. 14·5% protein basal diet+0·025% tryptophan+0·05% isoleucine
11. 14·5% protein basal diet+0·025% tryptophan+0·05% valine
12. 14·5% protein basal diet+0·025% tryptophan+0·05% lysine+0·05% isoleucine +0·05% valine

0·05% lysine, 0·05% isoleucine, 0·05% valine and a mixture of all three respectively.

A group of 600 light hybrid layers is being used in the trial which is still in progress. Each laying cage contains 3 birds and food and water are provided *ad libitum*. Records are kept of egg production, egg weight and food intake. The results presented in Table 3 are the means for the first 6 months of the experiment.

The egg production data show that diet 1 supported a reasonable level of egg production which was improved by a tryptophan supplement (diet 2). Diets 3 and 4, although calculated to be adequate in essential amino acids did not support a good level of egg production. Diet 4 supported a slightly better production than diet 3 suggesting the involvement of a specific amino acid deficiency, but the determined protein content of these diets (around 10·0%) suggests the depression of egg production was due to an insufficiency of non-essential nitrogen. The additions of tryptophan to the basal diet containing 14·5% protein each gave a good response indicating the inadequacy of tryptophan in this diet. There was no difference in egg production when diets 7 and

8 were fed, suggesting that the allowance had already been met at the lower level. Further amino acid additions did not lead to any improvement in production. Adding 0·05% isoleucine depressed production indicating the allowance was probably being exceeded.

There was no increase in egg weight following an addition of 0·025% tryptophan to diet 1. Egg weight was depressed when the essential amino acids were added to the 10·5% basal diet (diet 3). An addition of 0·025% tryptophan to this (diet 4) did not lead to such a marked depression. indicating again that a specific amino acid deficiency may have been involved as well as a shortage of non-essential nitrogen.

TABLE 3

*Mean productive performance (6 months)*

| Treatments | Mean egg production (hen/day) | Mean egg weight (g) | Food intake (g/bird/day) | kg Food per dozen eggs | g Food per g egg |
|---|---|---|---|---|---|
| 1 | 72·1 | 58·8 | 111·4 | 1·85 | 2·63 |
| 2 | 76·0 | 58·2 | 111·6 | 1·76 | 2·52 |
| 3 | 61·6 | 56·8 | 107·6 | 2·10 | 3·06 |
| 4 | 69·3 | 58·6 | 110·4 | 1·91 | 2·72 |
| 5 | 73·1 | 59·8 | 115·4 | 1·89 | 2·64 |
| 6 | 75·4 | 58·6 | 109·3 | 1·74 | 2·47 |
| 7 | 79·3 | 60·2 | 115·9 | 1·78 | 2·43 |
| 8 | 79·5 | 59·2 | 117·2 | 1·77 | 2·49 |
| 9 | 78·9 | 59·2 | 116·1 | 1·77 | 2·49 |
| 10 | 77·6 | 59·2 | 114·0 | 1·76 | 2·48 |
| 11 | 80·0 | 59·6 | 117·4 | 1·76 | 2·46 |
| 12 | 79·4 | 59·8 | 115·3 | 1·74 | 2·43 |
| S.E. | 1·15 | 1·04 | N.S. | | |
| L.S.D. | 2·51 | 2·08 | | | |

S.E.=Standard error
L.S.D.=Least significant difference; p. 0·05
N.S.=No significant differences.

Adding any amino acids to the 14·5% protein diet did not appear to affect the mean egg weight.

Voluntary food intake (expressed as g per bird per day) was relatively constant. It would seem therefore that the regulation of voluntary food intake in terms of the potential yield of energy from the diets upon oxidation was sufficient to over-ride any tendency to an increased food intake to compensate for a dietary amino acid deficiency or even any depression to protect against a potential surplus. Alternatively, in so far as a depression of voluntary food intake may be taken as an indication of a dietary amino acid imbalance, it is possible that the adult laying hen is less susceptible than is the young growing chick in such circumstances. A further explanation is that the amino acid imbalances were not sufficiently severe to cause a depression of voluntary food intake. Food intake expressed as kg food per dozen

eggs reflects the relative production, but when expressed as g food per g egg, rations 7 and 12 are seen to be the most efficient.

## *Discussion*

On the evidence of the data presented, it would appear that diet 7 (14·5% protein diet plus 0·025% tryptophan) is the most satisfactory. In view of the excellent production supported by this diet it is reasonable to assume that the amino acid levels in this diet approximate to the optimal allowances under these conditions. There may, however, be a surplus of some of the amino acids supplied without any adverse effect resulting.

Since Hill (1956) has shown that in terms of the energy yielding component there is a voluntary regulation of food intake it is necessary

TABLE 4

*Amino acid allowances for laying hens*
*(mg per bird per day)*

| | Combs (1961)[1] | Fisher (1960)[2] | Adkins, *et al.* (1961) | Current trial[3] |
|---|---|---|---|---|
| Lysine | 667 | 443 | 970 | 794 |
| Histidine | 233 | 139 | 397 | 391 |
| Methionine | 317 | 259 | 980 | 414 |
| Methionine & Cystine | 550 | 450 | — | 748 |
| Tryptophan | 150 | 123 | 245 | 167 |
| Phenylalanine | 567 | 376 | 490 | 759 |
| Phenylalanine+Tyrosine | 967 | — | 1372 | 1334 |
| Leucine | 917 | 508 | 1666 | 1415 |
| Isoleucine | 667 | 508 | 784 | 690 |
| Threonine | 484 | 500 | 686 | 736 |
| Valine | 717 | 520 | 931 | 771 |

(1) Production 80% Egg Weight 57 g Liveweight 1·8 kg
(2) Production 56% Egg Weight 50 g Liveweight 1·5 kg
(3) Production 80% Egg Weight 60 g Liveweight 1·8 kg

to define amino acid allowances as some function other than a proportion of the diet. The most convenient expression is the quantity of amino acid ingested per unit time, thus allowing dietary amino acid or 'energy' levels to be adjusted accordingly. On this basis, the amino acid allowances as provided by diet 7 have been expressed as intake in mg per bird per day and compared with other proposed values (Table 4). The amino acid intake in the recent trial is presented for a total food intake of 115 g per bird per day. The values of Fisher (1960) refer to a summation of the maintenance requirement and the needs for a 50 g egg. The values of Adkins, Harper and Sunde (1961) are actual intake values using diets based upon free amino acids. The figures of Combs (1961) are calculated for a 4 lb laying hen producing eggs at an average of 80% during the winter and a mean egg weight of 57 g.

The theoretical values of Fisher (1960) are in every case less than the values established by actual intake. The amino acid allowances determined in the recent trial relate to a mean production of 80%, a mean egg weight of 60 g and a liveweight of 1·8 kg (4 lb). The values of Fisher (1960) are based on a production of 56%, a mean egg weight of 50 g and a liveweight of 1·5 kg. The allowances determined in the recent trial are less than those of Adkins (1961). Adkins *et al* (1961), however, recognized that the values they found were probably in excess of the amounts required by the laying hen, but found it necessary to supply these amounts of amino acids to maintain optimal egg production with a free amino acid diet. The amino acid allowances calculated from the data of Combs (1961) are slightly less than the observed intake figures, but refer to a 57 g egg whereas the mean egg weight in the current trial was 60 g. The excellent level of production achieved by the birds fed diet 7 indicates that a good balance of amino acids had been achieved. However, it may be possible to achieve a further nutrient economy by adjusting the ratio of essential to non-essential amino acids. The encouraging performance of birds fed diet 2 suggests that an improvement in production might be achieved by a simultaneous reduction of all the essential amino acids whilst still retaining the same total level of protein. Alternatively certain individual amino acids are possibly present in excess of amounts needed by the laying hen. In particular the levels of histidine, leucine and threonine might advantageously be reduced to a lower dietary level. It is intended that subsequent experimental programmes will pay particular attention to non-essential nitrogen and amino acids that might be present in excess of optimum allowances.

It must be stressed that these results refer only to the first six months of an experimental programme. The main objective has been to maintain good egg production and use standard materials as the main source of amino acids. Until further information becomes available it is considered to be justifiable to propose that the amino acid intake of the birds fed diet 7 represents at least an adequate estimate of the optimal amino acid allowances.

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# 10

# AMINO ACID ALLOWANCES FOR TURKEYS

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*Synopsis*

The amino acid and protein requirements of starting, growing, and breeding turkeys have been reviewed. Absolute amino acid requirement data are extremely limited even for starting poults. Suggested amino acid patterns for turkey growth based on carcass analyses and chick amino acid requirements in combination with known turkey requirements are presented. These patterns have been used to calculate requirements for growing turkeys 8-24 weeks of age, and estimated amino acid allowances are presented.

Supplemental amino acids required for optimum performance on low- or normal-protein, corn-soya bean meal rations have been reviewed. It is found in such rations that methionine is the most severely deficient amino acid for young poults, followed by lysine and possibly arginine. During the growing period lysine becomes the most limiting amino acid followed by methionine and possibly arginine. Requirement data for these two amino acids and minimum protein levels during various growing periods are discussed.

Turkey breeders exhibited a wide tolerance towards variations in dietary protein and energy levels. In general, protein levels as low as 12% were satisfactory, hence 15-16% protein with metabolizable calorie/protein ratios of 75-80 should be at least adequate.

## *Introduction*

IN examining the listed amino acid requirements of turkey poults, it is found that for only seven of the thirteen essential amino acids is there an established requirement (see Table 1). For the remaining six there are only suggestions based on either chick requirement values, turkey carcass composition, or levels known to support good growth using natural protein sources. Further, no amino acid allowances are listed for either growing or breeding turkeys. This lack of information obviously limits discussion on absolute amino acid requirements of turkeys and especially of older turkeys.

To circumvent this problem to some degree the amino acid allowances for turkeys will be reviewed where known, but in addition, predicted or calculated amino acid requirements will also be presented. Furthermore, attention will be paid to supplemental levels of amino acids required for normal or low-protein starting and growing rations.

Since most of these studies have been conducted with corn-soya bean meal type rations, the basal dietary level plus the required supplemental levels of the amino acids under study give reasonable estimates of the dietary requirements, if a high availability from the natural ingredients is assumed.

This report will not emphasize factors which are said to affect the amino acid requirement of turkeys, e.g. essential amino acid availability, environmental temperature variations, and growth rate differences. Shortage of space, but more significantly, the lack of available information necessitates this. However, as implied above, amino acid availability would be relatively constant in most of the studies to be reported, since corn-soya bean meal rations have been almost exclusively used in turkey protein studies. Attention will of course be paid to energy-protein or energy-amino acid relationships, since the energy content of the ration markedly influences requirements by altering nutrient intake.

### *Protein and Amino Acid Requirements of Starting Poults*

The amino acid requirement estimations and studies with poults have taken one of the following forms:

1. The use of the amino acid composition of the turkey carcass as a means of estimating the amino acid pattern required for growth, with amino acid supplementation based on these levels. (Scott, 1963; also see Table 1).
2. The use of the poult's known requirements, plus the chick's amino acid requirements times the factor 1·4 for those amino acids whose requirements have not been determined for poults, as a basis for developing an amino acid pattern to be tested for its growth-promoting capacity in poults (Waibel, 1959; also see Table 1).
3. The supplementation of natural low-protein rations with free amino acids to give an amino acid pattern found in a mixture of natural proteins which, when added to poult rations, promotes optimum growth (technique used by Carter, Naber, Touchburn, Wyne, Chamberlin & McCartney, 1962; Fitzsimmons & Waibel, 1962; and Carlson, 1965). This method obviously is limited to the study of the more limiting amino acids, but gives the most practical and useful values.
4. The use of free amino acid mixtures based on the foregoing paragraphs 1, 2, or 3 but with amino acid levels adjusted on the basis of growth assays for individual amino acids plus an analysis of plasma amino acid values (Snetsinger, Britzman, Fitzsimmons & Waibel, 1964; Dunkelgod, Waibel, Snetsinger, & Sirny, 1965).

Many of the early protein and amino acid studies with turkeys were conducted by Kratzer and co-workers at California in the 1940s and

early 1950s and have been reviewed by Almquist (1952). More recently Waibel (1960) has reviewed later protein and amino acid studies; consequently this paper will deal primarily with reports which have appeared since 1960. A brief review of these earlier studies indicates that a 28% protein diet with a metabolizable calorie/protein ratio of approximately 40 gave optimum performance with Broad Breasted Bronze turkeys (Waibel, 1959; Atkinson, Kurnick, Ferguson, Reid, Quisenberry & Couch, 1957, among others).

Methionine was found to improve growth on low-protein practical diets in early studies (Bird, Marsden & Kellogg, 1948); later studies (Slinger, Pepper & Hill, 1953; and Waibel, 1959, among others) found that supposedly adequate 28% protein rations were deficient in methionine and these studies further defined the dietary levels of methionine required. Lysine also was shown to be a limiting amino in corn-soya bean meal, low-protein diets (Baldini, Rosenberg and Waddell, 1954); this was later confirmed (Fisher, Dowling and Maddy, 1956). In both studies small type turkeys were used and performance on the low-protein, amino acid supplemented rations equalled that on high-protein control rations.

Balloun and Phillips (1957) found that the protein level of starting diets could be lowered from 27 to 25% of the diet if the ration was supplemented with both lysine and methionine. Using high energy diets (15% added fat) Waibel (1959) found that methionine was the most limiting amino acid of corn-soya bean meal rations with 32% protein in the diet. At a 28% protein level both methionine and lysine were limiting.

These early reports may be summarized as follows: Turkey poults require a minimum of 28% protein in their diet with a metabolizable calorie/protein ratio of approximately 40. With corn-soya bean meal rations of the above protein level, growth responses to added methionine are usually observed. As the protein level is decreased to approximately 24%, lysine also becomes limiting.

Earlier reports had indicated that lysine and methionine supplementation of low-protein diets (20%) gave nearly optimum growth with small-type turkeys. Recently Fitzsimmons and Waibel (1962), using 20-24% protein, corn-soya bean meal rations, attempted to determine whether this could be repeated with the larger Broad Breasted Bronze or White poults from 0-6 or 0-4 weeks of age. In experiments with the 24% protein diet it was found that methionine was the principal limiting amino acid while the lysine intake appeared to be marginal. It seemed that 0·83% of sulphur-containing amino acids and 1·36% of lysine satisfied the poults' requirements—levels slightly under published requirements. For poults fed the 20% protein diet, methionine and lysine were the first and second limiting amino acids respectively; however, it was not possible, even with adequate methionine and lysine, to attain 'control' growth, indicating that other

amino acids were probably limiting. Supplementation with a combination of valine, glycine, phenylalanine and arginine gave small but non-significant growth responses. Balloun (1962) found that with a 24% protein diet containing 2200 productive calories per kilogram, Bronze males responded primarily to methionine additions. Lysine supplements gave slight responses while arginine-additions in the presence of adequate methionine and lysine gave small and inconsistent responses. A dietary allocation of 0·80% sulphur-containing amino acids was adequate, while arginine and lysine levels of 1·57% and 1·40% of the diet (calculated values) respectively appeared marginal.

Sherman, Donovan and Reynolds (1960), in a series of poult experiments, reported consistent responses to lysine on rations of different protein content and quality. Diets were based on corn-soya bean meal, corn-soya bean meal, and fish meal as well as other protein sources.

Attempts by Carlson (1961) to find the limiting amino acids of a 20% protein corn-soya bean meal ration indicated that the addition of 0·6% glycine on top of supplements of 0·35% L-lysine and 0·2% methionine resulted in a further growth improvement. Later Britzman and Carlson (1962) and Carlson (1965) could not substantiate the earlier response to glycine.

In verification of the later reports of Carlson, recent unpublished studies at Minnesota also indicated that poults 0-6 weeks of age do not respond to glycine additions made to 20% protein corn-soya bean diets made adequate in methionine and lysine. In the Minnesota studies arginine supplements have occasionally given marginal growth responses similar to those observed by Balloun (1962). In these studies supplemental levels of 0·36% methionine and 0·25% lysine were required for maximum gains; however, even with arginine and/or glycine supplementation it has not been possible in any of the tests to achieve the performance of poults fed the 28% protein control ration. It thus appears from these supplementation experiments that the correct balance of amino acids beyond the first two limiting amino acids has yet to be achieved.

From these supplementation experiments it is possible to obtain the requirement for three or four of the essential amino acids as well as some practical data on supplementation levels. However, of additional interest is the exact pattern of amino acids required for growth, *i.e.* the amino acid mixture which will support good growth but contain minimum levels of all essential amino acids.

Scott (1963) has indicated that the amino acid pattern found in turkey carcass protein closely compares (on the basis of percentage of protein) with the turkey's known amino acid requirements. These comparisons are presented in Table 1. Only the listed amino acid requirements for glycine, histidine, tyrosine and lysine differ markedly

from counterpart values in tissue, and only in the case of glycine does the tissue composition underestimate the requirement.

Waibel (1959, 1963*b*) has estimated the essential amino acid pattern or requirements of starting turkeys, using a combination of the independently determined requirements for the poult and, for those amino acids whose requirements are not known, an estimated value based on the chick's requirement times the factor 1·4. Snetsinger, Waibel and Fitzsimmons (1962), in developing a 'crystalline amino acid' diet for poults, found that an amino acid mixture based on the

TABLE 1

*The amino acid requirements of the turkey poult 0-6 weeks based on requirement data, estimated values, carcass composition and free amino acid diets.*[1]

| | National Research Council (1960) | Turkey carcass amino acid pattern [2] | Calculated requirements | | Amino acid diets | |
|---|---|---|---|---|---|---|
| | | | Scott (1963) | Waibel (1963) | Dean & Scott (1962) | Dunkelgod *et. al.*, (1963) |
| Arginine | 1·60 | 1·82 | 1·68 | 1·64 | 1·34 | 1·29 |
| Glycine | 1·00 | 0·84 | 1·68 | 1·03 | 1·96 | 1·73 |
| Histidine | ? | 1·40 | 0·70 | 0·43 | 0·40 | 0·70 |
| Isoleucine | 0·84 | 1·40 | 1·26 | 0·86 | 0·98 | 1·66 |
| Leucine | ? | 2·13 | 1·96 | 2·01 | 1·47 | 2·25 |
| Lysine | 1·50 | 2·52 | 1·46 | 1·54 | 1·20 | 1·71 |
| Cystine | 0·35 | 0·28 | 0·42 | 0·36 | 0·43 | 0·69 |
| Methionine | 0·52 | 0·73 | 0·56 | 0·53 | 0·43 | 1·04 |
| Phenylalanine | ? | 1·04 | 1·12 | 1·00 | 0·61 | 1·57 |
| Tyrosine | ? | 0·42 | 0·98 | 0·72 | 0·55 | 1·10 |
| Threonine | ? | 1·12 | 1·12 | 0·86 | 0·80 | 1·24 |
| Tryptophan | 0·26 | 0·25 | 0·28 | 0·27 | 0·28 | 0·45 |
| Valine | ? | 1·43 | 1·40 | 1·15 | 1·00 | 2·01 |

[1] Expressed as a percentage of the ration, energy level of which is approximately 2750 metabolizable cal/kg.

[2] Based on the pattern and quantity of amino acids that would be furnished by 28% protein from turkey carcass protein.

amino acid pattern of chicken egg white protein gave greater gains than a mixture based on the above combination of requirement data plus calculated values. The best growth obtained was however, still quite low—namely, 6 g/poult/day.

Later experiments at Minnesota (Dunkelgod, Waibel, Snetsinger & Sirny, 1965) compared free amino acid mixtures, based on either the Illinois chick free amino acid mixture (Dean & Scott, 1962) or the Minnesota egg-white protein-based mixture. Results indicated that the pattern of amino acids found in the Illinois mixture was far superior to that of the Minnesota mixture in supporting growth of poults from 7-21 days of age. A comparison of the amino acid patterns found in these mixtures can be seen in Table 1.

It is difficult to take from Table 1 a consensus value as the actual requirement for any given amino acid, since there is wide variation in suggested levels or levels shown to promote satisfactory growth. Secondly, there is also the question whether the requirements determined with mixtures of amino acids will apply when the amino acids are furnished by natural proteins. Amino acid availability must be taken into account with natural protein sources. There is also the question of individual amino acid absorption rates. Do the rates show marked differences between free amino acid diets, where all the amino acids are ready to be absorbed immediately, and natural protein diets, where the amino acids are possibly released at different rates in the digestive process and some may thus be absorbed more slowly than others? That there are differences between free amino acid diets and natural protein mixtures of equivalent amino acid content is shown by the fact that an amino acid mixture based on egg protein (Snetsinger, Waibel & Fitzsimmons, 1962) supports only low growth, whereas virtually the same amino acid mixture derived from a mixture of soya bean, corn, fish meal, blood meal and yeast supports excellent growth (Thayer, Dunkelgod & Benton, 1961).

Obviously, answers to these questions are not yet available and because of the expense of free amino acid diets and relevant studies they will be slow in coming. In the Minnesota studies the highest gains have been 16-17 g/poult/day. This weight increase was still approximately 20% lower than achieved with a natural diet. Plasma amino acid values were utilized as an additional guide to amino acid adequacy; the plasma values for the amino acids studied, namely histidine and phenylalanine, have verified the growth data as to requirement and appear to offer a valid criterion for amino acid adequacy.

### *Protein and Amino Acid Requirements of Growing Turkeys*

It has been only in recent years that attention has been paid to the amino acid requirements of growing turkeys and this again has been primarily in an attempt to establish the limiting amino acids of corn-soya bean meal rations.

*8-12 Week Period.* Early work showed a requirement of approximately 20% protein with a metabolizable calorie-protein ratio of approximately 55 to 60 as satisfactory for turkey performance during this period.

In Minnesota studies (unpublished data), turkeys fed 16% and 18% protein corn-soya bean meal rations with combinations of methionine, lysine, arginine and glycine supplements, were compared to control groups receiving 22% protein. The addition of 0·1% methionine and 0·2% lysine or 0·2% methionine plus 0·4% lysine resulted in marked growth responses when added to a 16% protein diet; however, turkey performance was not equal to that of the control group. Addition of

arginine or glycine in addition to the methionine and lysine supplement gave either little or no non-significant growth responses. Diets with 18% protein were improved by the addition of methionine but not lysine; however, growth of turkeys fed these diets was again not equal to that of the 22% protein controls.

In the studies of Carter, Naber, Touchburn, Wyne, Chamberlin & McCartney (1962), it was found that the supplementation of a 17% protein corn-soya bean meal ration with 0·2% of methionine and 0·2% lysine gave performances equal to control (20% protein) fed turkeys. At 14% protein, lysine and methionine supplementation alone or in combination, was not sufficient to give control growth. Lysine was the most limiting amino acid at 14% protein, whereas at 17% protein, methionine was more limiting than lysine. The sulphur-containing amino acid requirement with 2025 productive calories/kilogram is calculated not to exceed 0·73% of the diet, whereas the lysine requirement does not exceed 1·13%. These values agree closely with those suggested by Waibel (1963*a*).

Balloun (1962) found that a lysine minimum of 5% of the protein was required during the 6-10 and 10-12 week periods for large white turkeys. The initial response (0-6 weeks) to methionine diminished as the turkeys became older. Arginine again gave a small growth response.

It is important to note that large sex differences in responses to amino acid supplementation become apparent, particularly during the growing period. Carter *et al.* (1962) and Balloun (1965) point out that the responses to methionine and lysine are large with growing males, but little responses are seen with females. To achieve the most economic gains it is important to separate the sexes and carry out separate feeding programmes so as to obtain not only the growth desired but the finish as well.

*12-18 Week Period.* Experiments conducted at Minnesota with heavy-type males from 14-18 weeks indicated a marked response to additions of 0·15% methionine and 0·40% lysine to a 13% protein corn-soya bean meal diet (productive calorie/protein ratio of 78). Arginine and glycine supplementation was of no benefit. Growth of turkeys was not equal to that shown by 20% protein control groups; however, turkeys fed a 15% protein ration (C/P of 66) with the addition of 0·1% methionine and 0·2% lysine equalled the performance of those given the control ration. This level of protein shows a considerable reduction from practical levels of protein of approximately 19% in diets that usually are fed during this period.

The work of Balloun (1962) indicated that a value for lysine of 5% of the protein was essential during the growing period for optimum growth. The data of Carlson and Guenthner (1963) substantiate the finding for this period; however, they found after 20 weeks that lysine could be as low as 4·1% of the protein and still permit optimum weight

gains. In contrast to other reported studies, significant growth responses to lysine additions were observed with females as well as males. The minimum lysine intake which permitted optimum performance was 5·2 and 4·9% during the periods from 13-16 and 17-20 weeks respectively. Kratzer, Davis and Marshall (1956) had indicated a slightly lower value with lysine at 4% of the protein being suggested as the requirement for this period. Pepper and Slinger (1955) in an earlier study indicated that the methionine requirement during this period was 1·47% of the protein.

*18-24 Week Period.* In Minnesota studies, corn-soya bean rations with protein levels of 10% (productive C/P of 140), 12% (C/P of 85) and 14% (C/P of 71) were compared to a 17% protein control (C/P of 57). The 10% and 12% protein diets were inadequate for growth while the 14% with no supplemental amino acids permitted optimal performance. In one year the 12% protein diet with 0·3% lysine added also permitted control performance, although the results were not repeated in the following year. Arginine, glycine or methionine additions to the 12% protein diets were not beneficial.

As indicated previously, Balloun (1962) observed responses to lysine during this period if the lysine level fell below 5% of the protein, whereas methionine at 2% of the protein was required. Carlson and Guenthner (1963) suggested that the lysine requirement was as low as 4·1% with fast-growing males during the period from 20-24 weeks. Field experience indicates that 14-15% protein levels without amino acid supplementation and with Metabolizable Energy calorie-protein ratios of approximately 100 for the males and 105 for the females will promote satisfactory performance during this period.

It is obvious from the foregoing that lysine and methionine are the most limiting amino acids of corn-soya bean meal growing diets, with lysine being primary in the late growing periods while the methionine inadequacy occurs fairly early. This reversal occurs not so much because of a dramatic change in requirements but because of a switch, as the protein level is lowered during the growing period, from soya bean meal as the dominant source of protein to greater reliance on corn as the protein source (see Table 2). Availability of amino acids, notably lysine, from corn versus soya bean meal may also influence this general change.

It is also apparent from this discussion that data on amino acid requirements for growing turkeys are extremely limited. Outside methionine and lysine inconsistent responses to arginine and glycine have been reported, but no other amino acids have been thoroughly tested. Thus to list any amino acid requirements is not too meaningful; however, in order to have some basis for ascertaining the limiting acids, the calculated requirements based on starting poults' requirements (none too accurate in themselves) and adjusted for the protein and energy changes required for older turkeys are presented in Table 2.

The confounding of the dietary amino acid changes with simultaneous energy changes adds to the difficulty of establishing precise amino acid requirements. One means of increasing the meaning of these studies is to establish the requirements on a daily nutrient intake basis. A series of papers from Oklahoma State University (Thayer, Dunkelgod & Benton, 1961; Dunkelgod, Gleaves, Tonkinson, Thayer & Sirny, 1961) has developed feeding standards which are altered weekly to meet

TABLE 2

*Estimated amino acid requirements of growing turkeys*[1]

| Age in weeks: | 8-12 | 12-10 | 16-20 | 20-24 |
|---|---|---|---|---|
| Percentage Protein | 22 | 20 | 16 | 14 |
| M.E.* cal/kg | 2700 | 2790 | 2900 | 2990 |
| Arginine | 1·29 | 1·17 | 0·94 | 0·82 |
| Arginine[2] | 1·16 | 1·07 | 0·93 | 0·80 |
| Arginine in corn-soya diet | 1·18 | 1·26 | 1·04 | 0·84 |
| Glycine | 0·81 | 0·74 | 0·59 | 0·52 |
| Histidine | 0·34 | 0·31 | 0·25 | 0·22 |
| Isoleucine | 0·68 | 0·61 | 0·49 | 0·43 |
| Leucine | 1·58 | 1·44 | 1·15 | 1·01 |
| Lysine | 1·21 | 1·10 | 0·88 | 0·77 |
| Lysine[2] | 1·12 | 1·00 | 0·87 | 0·75 |
| Lysine in corn-soya diet | 1·08 | 0·97 | 0·78 | 0·63 |
| Methionine | 0·42 | 0·39 | 0·30 | 0·26 |
| Methionine[2] | 0·40 | 0·37 | 0·30 | 0·26 |
| Methionine in corn-soya diet | 0·36 | 0·34 | 0·30 | 0·28 |
| Cystine | 0·28 | 0·26 | 0·21 | 0·18 |
| Phenylalanine | 0·79 | 0·71 | 0·57 | 0·50 |
| Tyrosine | 0·57 | 0·51 | 0·41 | 0·36 |
| Threonine | 0·68 | 0·61 | 0·49 | 0·43 |
| Tryptophan | 0·21 | 0·19 | 0·15 | 0·13 |
| Valine | 0·90 | 0·82 | 0·68 | 0·58 |

[1] Based on Waibel's (1959) estimated amino acid requirements of starting poults with adjustments made for the lower protein requirement of the older turkeys.
[2] Estimated requirement values of Balloun (1965).
* ME=Metabolizable Energy.

the changing nutrient requirements of the turkey. These standards emphasize not only a balance between energy and protein but all other nutrients as well. Through such weekly alterations in ration, the time needed to reach market weights was shortened by three weeks and substantial reductions in feed intakes occurred. Additional papers in this series (Dunkelgod & Thayer, 1961; Thayer *et al.*, 1961) further emphasize the necessity for separating the sexes for optimum performance, since lower protein and high energy intakes are required for optimum gains by the hens at a given age.

The formulas in these studies rely upon blending high-quality protein sources which accounts in part for the success of the Oklahoma feeding programmes. At present the use of these formulas with all the changes required is virtually prohibited on economic grounds. However, the basis of the programmes is sound; it points the way toward better balance of amino acids through amino acid supplementation and judicious choice of proteins, and it indicates a trend toward a greater number of formulas to match changing amino acid and energy requirements.

### *Turkey Breeders*

Limited data are available on the protein requirements of turkey breeders. Carter, Wyne, Chamberlin and McCartney (1957) studied breeder diets having respectively 16% and 18% protein in combination with 1760, 1980 and 2200 productive calories per kilogram. Only small differences between treatments were seen; however, 18% protein diets brought about slightly better hatchability and fertility, and body weight loss was slightly less than on the 16% diets.

TABLE 3

*Relationship of protein requirements of turkeys to dietary energy content of ration and age*

| Metabolizable energy | Age in weeks | | | | |
|---|---|---|---|---|---|
| | 0-8 | 8-12 | 12-16 | 16-20 | 20-24 |
| | Percentage of protein in diets | | | | |
| cal/kg | % | % | % | % | % |
| 2640 | 28·0 | 21·5 | 18·5 | 14·3 | 12·4 |
| 2750 | 29·0 | 22·4 | 19·3 | 14·9 | 12·9 |
| 2860 | 30·0 | 23·3 | 20·0 | 15·5 | 13·4 |
| 2970 | 31·0 | 24·1 | 20·8 | 16·1 | 13·9 |
| 3080 | 32·0 | 25·0 | 21·6 | 16·7 | 14·4 |

Robblee and Clandinin (1959) used 15% and 17% protein diets in a factorial design with energy levels of 1540, 1740 and 1940 productive calories/kilogram and found no differences by any performance criteria. Jensen and McGinnis (1961) used protein levels as low as 10% and found no depression in performance of turkey breeders. Atkinson *et al.* (1960) studied higher protein levels in breeder diets and found that 22% gave the highest egg production. No effects of protein level on hatchability or fertility were seen.

Waibel (1963*a*) reported that protein levels of 13%, 15% and 18% in combination with productive C/P ratios of 106, 90 and 73 respectively did not differ by any production criteria. In an experiment utilizing Jersey Buff or large white turkey breeder hens, increasing the energy content of rations containing 14·5% or 16·5% protein had no effect with either breed on hatchability or fertility (Anderson, 1964). For the

Jersey Buff turkeys the energy content of the ration apparently directly controlled energy (nutrient) intake. For the large white turkeys calorie consumption increased at the lower protein intake when the caloric content of the diet increased. It was suggested that an associated increase in egg production was due to improved protein utilization in the presence of fat.

Owings (1963) observed that lysine additions of 0·2, 0·4 and 0·6% to a 15% corn-soya bean meal ration resulted in improved fertility and hatchability of eggs from breeder hens receiving the test diets. The lysine supplements had no effect on egg production. Confirmation from other laboratories of these results is still awaited. How to explain these results is not immediately apparent and it is confusing that amino acid deficiencies with chicken breeders tend not to reduce fertility or hatchability but to stop production.

The review indicates that turkey breeder stock are for the most part relatively insensitive to major protein or energy changes, as evidenced by their ability to maintain egg production on protein levels as low as 10% and tolerate productive C/P ratios of 56 to 100. Another contributing factor to the above variation is undoubtedly the environmental temperature under which the studies were conducted (southern versus northern United States). It would seem that with typical corn-soya bean rations, 15-16% protein with metabolizable C/P ratios of 75 to 80, should be at least adequate. These quantities can serve as useful guides until additional critical studies are conducted.

This review of the amino acid allowances of turkeys has re-emphasized to the author how extremely limited is the available data. However, studies designed to establish the absolute amino acid needs of turkeys are increasing. Additional research will further be encouraged by reduction in amino acid prices so as to permit greater use of amino acid supplements in practical rations. The use of diets containing free amino acids will permit the discovery of amino acid patterns giving accurate estimates of the poult's requirements of the essential amino acids. These approaches will soon permit the removal of a number of the question marks that presently exist.

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# DISCUSSION ON PART III

*Mr P. Cooper (Colborn Vitafeeds Ltd., Canterbury):* We have never successfully overcome the problem of weighing shell-less and soft-shelled eggs. Can anyone suggest how to deal with this?

*Professor D. C. Snetsinger (University of Minnesota):* We have, I suppose, largely ignored this question. However, an adjustment can be made on the basis of the percentage of the total weight due to the shell in a normal egg.

*A voice:* Could I just say that I'm much more interested in how you pick them up. (Laughter).

*Dr R. Roberts (J. Bibby & Sons):* Professor Snetsinger suggested that he had misgivings about the acceptability of feeding natural protein together with synthetic amino acids. Would the analysis serially, (that is with time) of plasma amino acid levels, reflect any peaks in the synthetic additions relative to the natural protein sources?

*Professor Snetsinger:* Yes, I think it would, but we need experiments to determine how much. The use of isotopically labelled natural protein or free amino acid mixture would give you the same result. Jones at Rochester is, I believe endeavouring to incorporate arginine in casein, using an udder that has been removed from the cow. Differences in absorption rates might be found this way.

*Dr H. Temperton (National Institute of Poultry Husbandry):* In the turkey breeding experiment that Professor Snetsinger quoted, it struck me that the levels of egg production and of hatchability of fertile eggs were extremely low. I wonder whether Professor Snetsinger can account for this, and whether this may have been a restrictive factor thus reducing the confidence one should place in the different protein levels. The highest level of hatchability of fertile eggs was only 63% and the general level of production was in the 50's.

*Professor Snetsinger:* Definitely the hatchability was lower than we like to see. I am not so sure about egg production; this was a 20-week experiment and egg production over that period was reasonable. Professor McGinnis can probably tell you about the experiments he has conducted in the past in which extremely low levels of protein have been shown to be equally effective as 18% protein commonly used in the field. I just don't think that we have as yet had a test low enough in protein to have depressed egg production in our experiments.

*Professor J. McGinnis (Washington State University):* I would be more inclined to associate the low hatchability with the particular strain or breed of bird. Was this your large white bird? Strain differences would probably explain the low hatchability experienced by quite a lot of people; we would ourselves like to see it higher. As I recall, the figures we obtained on hatchability with the Broad Breasted Bronze

type of bird (smaller) were in the 80's on fertile hatch at 10% protein. I have a very strong feeling that the level of protein being fed to turkey breeders is often far in excess of their actual requirements. I would agree with Prof. Snetsinger, that egg production over that length of time with his kind of bird, is not too bad.

*Dr C. Calet (Jouy-en-Josas):* May I make two points. The first is about the very complete and interesting paper of Professor Combs: it deals with the effect of free amino acids on feed consumption. We have carried out an experiment with purified diets, which were deficient in two amino acids. The first missing amino acid is methionine and the second one glycine; when the first limiting amino acid was added, of course, weight gain and feed intake rose; when glycine alone was added, the diet still lacking methionine, weight gain did not get better but feed intake was much improved. The lack of effect of lysine supplementation, without methionine, on weight gain confirms that glycine is not the first limiting amino acid. The concomitant great increase in feed consumption suggests that the amino acids have a specific influence on appetite.

The second point concerns the dietary protein level for layers and their needs for methionine. In view of Professor Combs' results with chicks, experiments were conducted in our laboratory to determine the influence of the protein level of the diet on the feed intake of the laying hen.

Laying hens kept in individual cages were given 10 diets made up with two different kinds of proteins (high and low biological value) and five protein levels (13 to 30%). The remaining part of the diet contained starch, cerelose, minerals, oil and vitamins. Methionine was the limiting factor of the diet with low biological value proteins. It appeared generally that neither the protein level, nor the biological value influences the mean feed consumption: 117g per day per hen, in every case. There were, however, important variations of feed intake between individuals, and consequently the sulphur amino acid intake was very different between animals and between diets.

When the amount of sulphur amino acid ingested is plotted against the total amount of egg output, two regression lines are obtained, intersecting for the value of methionine+cystine requirement. From this graph it appears that egg production is a linear function of sulphur amino acid intake as long as the requirement is not satisfied; beyond this requirement value, sulphur amino acids in excess have no improving effect. It shows equally that laying is independent of the way the sulphur amino acid requirement is met. From a practical angle it might be pointed out that Heisdorf-Nelson hens receiving a diet which contains only 13% of high biological value proteins have an excellent egg production (80% laying rate).

Moreover it appears from these results that the notion of protein level *per se* is not accurate enough to define animal nitrogen requirements.

*Professor G. F. Combs (University of Maryland):* With regard to protein level we were concerned about the possibility of high levels of dietary protein having an effect on voluntary feed intake and feed conversion in the laying hen. In examining data from previous studies which involved various protein levels but only amino acid adequate diets, hens which received the higher protein levels generally consumed the least feed energy and exhibited the best feed conversion in five or six different trials. There appeared to be a continuous but slight reduction in calorie uptake as the protein level was elevated above that required for optimal egg production.

We then conducted a study in which the protein level was raised from 12% to 18% by increments of 1½%. The 13½% diet supplied 100% of our estimated amino acid needs of the layer. In this series of diets, amino acid quality of the protein remained the same. Another series of diets, ranging from 13½ to 18% protein, was used except that the lysine and total methionine content was kept constant as the protein level was raised; up to 6·7% hydrolysed feather meal was added for this purpose. During the first 11 weeks of this test the same type of differences occurred, with a 3% reduction in caloric uptake. After 21 weeks, however, there was no significant difference. We have had reports also that pullets which come into production in the spring will reduce their feed intake and egg size if high protein diets are fed during high ambient temperature periods.

*Professor D. Lewis (Nottingham):* In relation to what Dr Calet said, the method of expressing the requirement for amino acid is difficult to decide and I am sure that in the long run it is more realistic to visualize it in terms of egg production. But we have felt that we ought to express it per day, perhaps only because we haven't the courage to decline to give the bird any protein, even on the day it did not lay an egg. What are Professor Snetsinger's views on the use of synthetic amino acids and particularly of racemic mixtures. With a mixture DL, the D part could exert a toxic effect and, secondly, there is variability in its conversion to the L form. Hence there are two problems. How much then of D-form does one count? The uncertainty of the range of amino acids made us feel that with synthetic amino acid mixtures, unless they are exclusively in the L forms one never really knew exactly how much nutrient is being added. The precision is thus not quite what it seems to be.

*Professor Snetsinger:* I certainly agree with Professor Lewis's misgivings about DL mixtures. Where supplements consist of DL valine or DL isoleucine or some other amino acid it is assumed that none of the D is available. This assumption has been made too often. We have conducted some experiments on D versus L amino acids using a free amino acid diet. For valine I would say that something like 25% of the D form was available. By mistakenly treating it as unavailable and in view of a possible valine-leucine-isoleucine type of antagonism,

you could have problems and not know about them. With phenylalanine about 25-30% of the D was available compared to the L, again complicating the situation. In our experiments, we always used L-forms except in the case of methionine. The issue concerns all types of nutrition work and it would be better to make some judgment about it rather than ignore it completely. This applies especially to valine and methionine where we do have some information. We are conducting some experiments at Minnesota on germ-free animals using the available D amino acids. We shall then try to decide to what extent the D amino acid is made available, by microbial conversion with the normal gastro-intestinal microflora.

*Professor Combs:* May I be permitted a brief comment about microbiological conversions that might occur? In work at Maryland (Anderson, Combs and Briggs, 1950) DL and L tryptophan were fed to chicks and the conversion to niacin studied. In this work the carbohydrate of the diet was varied and in some diets sulfasuxidine was used to reduce microbial conversion of the D form to the L form. We found no conversion when sulfasuxidine was added at the 2% level, while a starch diet without sulfasuxidine gave 100% conversion. This is more than just a metabolic difference, since under certain conditions, bacteria may convert D amino acids to the L forms and materially affect the metabolically functional level of any amino acid where DL mixtures are used.

*Dr G. D. Rosen (London):* I would like to offer two comments and some information relevant to Professor Snetsinger's paper. We can confirm in typical U.K. mixed feeds for turkeys, amino acid responses (lysine and methionine) on high (27%) and low (21½) protein rations. Our results for the early stage of growth (0-3 weeks) ran very much in parallel with his. We also had obtained results in one test in which the high protein diet proved little better than the low protein because the fish meal used at 12½% in the formula turned out on subsequent testing to be of exceptionally low quality.

Professor Snetsinger referred to one cogent reason for gaps in our knowledge of turkey nutrition as compared with the broiler—namely, the high cost of turkey experiments. It is therefore of interest to report that the previously mentioned *T. pyriformis* assay, applied to the high and low protein turkey feeds, with and without amino acid supplements, revealed statistically significant differences in the protein quality of the feeds which correlated very well with turkey feeding data. The protozoal tests were completed in four days, much of which was incubation time.

*Mr W. R. Muir (Glaxo Laboratories Ltd.):* I would like to ask Dr Calet whether he carried out his experiments over a rather narrow range of egg production when he arrived at a definite figure, expressed as the amount per gram of egg, for the methionine requirement of the laying hen. The point of this question is that there is a methionine require-

ment for maintenance and perhaps a four times greater requirement for the production of one egg, so that one would expect the total methionine requirement expressed as a quantity per gram of egg to vary with the level of egg production. If Dr Calet made his observations within narrow limits of egg production, I would expect him to obtain a fairly constant figure for the methionine requirement, but I would prefer to see separate statements of the methionine requirement for maintenance and for the production of one egg.

I would also like to see methionine requirement expressed in terms of definite quantity. We could then express it as a percentage of the ration which would vary with the level of energy in the ration.

*Dr Calet:* Of course the first regression line does not pass through the zero of the co-ordinates.

*Mr W. R. Muir:* Yes, I understand that perfectly, but it still puzzles me why the methionine requirement should be fairly constant per gram of egg when the maintenance requirement for protein is of the order of 25% of the total protein required for 100% egg production.

*Mr T. R. Morris (Reading):* In the paper by Taylor and Lewis I notice that no standard errors were quoted for rates of lay and egg weight. Doubtless, these are interim results and the information will appear in the final report. That's one point. A second is about the logic of the argument, that this kind of experiment can lead to estimates of allowances for a whole range of amino acids. As I remember the diets, there was a graded level of tryptophan, a series of diets with different levels of tryptophan, which probably led to a rather tiny estimate of the tryptophan requirement. But for most other amino acids, and particularly for histidine and leucine which are probably present in great excess in this kind of practical diet, surely it is very misleading to calculate what is in the diet, which happens to be the most successful in this kind of experiment, and then turn round and say these represent estimates of the allowances for laying birds. Surely this procedure cannot give any critical estimate of the amount of an amino acid such as histidine, which is presumably not limiting in any of these diets—for example, the requirement for histidine of a laying bird. To set down a list of values as though one had estimated these allowances is, I think, misleading.

*Professor D. Lewis:* With regard to the first point raised by Mr Morris, there is a very simple answer, namely the amount of data that can be placed on a slide. To have added any statistical analyses to the matter on the slide would have been, I think, impossible. The information is, however, available and will be published (see page 140).

As far as the second point is concerned I think Mr Morris is quite confused about objectives. The need as I see it is to define a set of allowances that makes maximum egg production possible. If this does not happen, or something close to it, the information is valueless.

In establishing amino acid allowances it is necessary to select one

of two basic approaches: either to attempt to build up an amino acid mixture that supports adequate production or to take a practical system that does achieve it and steadily pare off any surpluses. The work that was reported fell into the latter category. Though in the tentatively proposed allowances there are clearly certain amino acids which have been given a value that is excessively high, it is at least possible to state that good egg production is supported. With the other approach, tentative proposals are in practice useless. Until satisfactory egg production can be demonstrated, there is no merit in formulating to any proposed standards. Although Mr Morris is no doubt referring to an ideal situation, one occasionally has to come down to earth and put forward a practical proposition.

The approach in the trials reported is to demonstrate satisfactory egg production at, say, 14·5% protein and then to drop the level to 12·5% and build up anew by appropriate amino acid supplementation, to satisfactory production. When this is achieved it is possible to repeat the exercise—dropping the protein level to, say, 10·5% of the diet—until the situation is reached when a decrease in the level of any part of the protein component fails to maintain the production standard that has been set. This approach has the further merit of not being totally dependent upon synthetic amino acids. Even during the earlier part of such a programme, as is now the case, it is possible to put forward a set of allowances that will allow normal productive capacity to be achieved. It is clearly acknowledged that there are still surpluses to be pared off and I would agree that to regard the allowances as physiological requirements would be totally misleading. But we claim that if they are followed egg production will not suffer.

*Professor Snetsinger:* I wish to make one additional comment on D-amino acid utilisation. Recently Tipton *et al.*, 1965 (*Poultry Sci.*, 44: 1422) presented data indicating that D-methionine was significantly more effective than L-methionine for growth promotion and feed efficiency in broiler rations. The relative values were approximately 105% for D-methionine versus 100% for L-methionine. This is the first positive report of this type I have seen. However, the data of Guttridge and Lewis, 1964 (*Br. Poult. Sci.*, 5: 193-200) also tends to give some indication that the D-isomer of methionine was more effective than the L-isomer. Relative potencies in their chick experiments were 106% for DL-methionine versus 100% for L-methionine. I wonder if Professor Lewis might comment on whether he feels his experiments indicated a true difference between the two isomers.

*Professor D. Lewis:* In that respect I never really dreamt that the D form was any better than the L. There was an indication given of the potential experimental error in that trial, which I think was well above 5% and I still assume that the D is somewhere below the 100. I think the actual record of 105% must be only fortuitous.

*Professor R. A. Morton (Liverpool):* In connection with D and L

problems, I am worried by two logical difficulties. May I just take an example, the problem of D and L tocopherol. Experimentally there is no doubt that D-tocopherol is about four times as potent as L-tocopherol by a great variety of animal tests. But if you compare the two *in vitro* they are equally potent. Now this means that a biological response is caused by two properties, chemical structure and optical activity, one of which has nothing to do with the final potency. Provided its D and L forms are active *in vitro*, in this case as an antioxidant, you are left with the 'transport' problem that the D form gets to the cellular site of action and the L form does not, or gets there very inefficiently. But with D and L amino acids we do know that in most cases only the L form will be incorporated in the protein of the egg, or in the protein of the tissue of the animal. There is therefore a question why the unnatural isomer should be any good at all. We have to assume, then, that there is some conversion but we have no right to assume that the conversion will have any standard efficiency as applicable to one amino acid as to another. We are left in a state of uncertainty about the *a priori* position of the biological activity of amino acids and particularly of unnatural (D-) amino acids.

*Professor Brown (Belfast):* I would welcome clarification of a point raised by Mr Morris in relation to the laying spasm described by Mr Taylor. In his calculations had he allowed for the utilization of the individual amino acids? If so, was there any indication, in view of the rather low levels of total protein, of a change in body weight of the fowl? The purely factorial method on diets similar to his basal diet would lead us to a figure of about 13½ grams of protein per day on 70% production. Are there any compensating effects in an experiment of this type?

*Professor Lewis:* In regard to availability, we made no assumption. In arriving at allowances and deciding values that are usable, the calculations are generally made without taking availability into account The convention is merely to pay some attention to it when it deviates below a normal level, a practice that is not by any means unique for this trial. On the second point of weight gains, as far as I am aware they weren't substantial.

*Dr B. R. Taylor (Nottingham):* In the first trials conducted last year the birds started off at approximately 1·73 kg and finished up at approximately 1·9 kg. This year they started off at more or less the same weight. As they were of the same strain—Maxilays (?)—in both cases, we may assume that they will attain the same weight at the end of the laying year, of just over 1·8 kg. In other words, on these diets they are gaining weight. Whether this increase represents protein or fat is not known, but this may emerge when at the end of this year I hope to do some carcass analyses.

*Dr C. G. Payne (Nottingham):* Professor Combs mentioned a zone of thermo-neutrality for laying hens. I seem to remember Professor

Romijn at the last conference suggesting that the laying hen doesn't have a well defined zone of thermo-neutrality. In fact the temperature of hyperthermal rise and the lower critical temperature are probably one and the same thing i.e., just one point rather than a flat zone of minimum basal heat output. Professor Lewis suggested that a further step may be to reduce protein levels at a subsequent stage down to 8·5%. I would suggest that another alternative would be to increase the temperature under which these birds are kept and thereby reduce the voluntary feed intake by 20% to 25% as required. We have kept hens at 60° and 85°F for a production period of 40 weeks. During the first month an average of about 20% came into lay. In the next 36 weeks the hens kept at 85° on a fairly well balanced ration have averaged 85%, whereas those kept at the cooler temperature have been slightly inferior at about 81% over that period. The consumption was about 115 grams at the low temperature and about 90 grams at the high temperature. I would suggest that increasing the temperature and getting a reduction in food intake may be a method by which one can get at the problem of amino acid balance quite effectively.

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# 11

# RELATIONSHIP OF DIETARY AMINO ACIDS TO THE OTHER NUTRIENT COMPONENTS OF POULTRY DIETS

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*Synopsis*

Utilization of protein and amino acids by poultry is affected by a number of different factors such as energy level, type of carbohydrate in the diet, ratio of essential and non-essential amino acids and presence of non-protein nitrogen. Even though it is commonly felt that non-protein nitrogen is of little significance in the nutrition of birds, there is ample evidence indicating that under carefully defined conditions, non-protein nitrogen supplied in the form of urea or ammonium salts can be utilized in lieu of non-essential amino acids.

Evidence is beginning to accumulate showing that non-protein nitrogen can be utilized by birds to meet a part of the dietary nitrogen needs when practical diets are employed.

## *Introduction*

NUTRITIONISTS have traditionally relied on products or feed ingredients containing protein to meet the amino acid requirements of poultry. In addition to relying on natural proteins to supply both essential and non-essential amino acids in poultry diets, investigative techniques used in determining specific amino acid requirements have doubtless obscured many interesting facts and potentialities for improvement and economy in supplying the nitrogen needs of poultry. Even though we have known for many years that ruminant animals are not the only ones that can make effective use of simple or non-specific sources of nitrogen such as urea, ammonium salts, etc., little effort has been directed at practical application of results showing that poultry, as well as other simple-stomach animals, can effectively utilize this type of combined nitrogen to meet a very significant part of the dietary protein needs. It would appear from an examination of the published literature, along with a considerable amount of unpublished data from our own laboratory, that we should no longer overlook the very great possibility of using significant quantities of non-protein nitrogen towards meeting the protein needs of the poultry industry. Also, more attention should be paid to the possibility that requirements of specific

essential amino acids by poultry of different types and ages may be modified or influenced by the presence of other amino acids in the diet, be they essential or non-essential. Some of the evidence we have obtained recently with laying hens might suggest the desirability of including a modest level of non-specific nitrogen in the diet.

It is appropriate at this juncture to review some of the published literature showing the specific utility of forms of nitrogen other than protein and amino acids for non-ruminant animals. A recent publication by Kies, Williams and Fox, (1965*a*) showed that human adults fed a diet based almost entirely on corn utilized the nitrogen in diammonium citrate and glycine to maintain a strikingly positive nitrogen balance. They concluded that the first limitation of corn, as far as nitrogen is concerned, is total or non-specific nitrogen as distinct from specific amino acid deficiencies. Kies, Shortridge and Reynolds (1965*b*) in another report showed that nitrogen retention of humans was improved by increasing the nitrogen intake, using a mixture of diammonium citrate, glutamic acid and glycine, while the essential amino acid intake was held constant.

Swenseid, Harris and Tuttle (1960) showed that a mixture of diammonium citrate and glycine was as effective as a mixture of non-essential amino acids in promoting nitrogen balance of humans. Snyderman, Holt, Dancis, Roitman, Boyer and Balis (1962) showed that essential amino acids are not the first limiting nitrogen factor for growing infants fed milk protein. Increasing the intake of nitrogen by using urea or glycine, restored nitrogen retention of infants to normal. These workers also demonstrated, using urea or ammonium chloride containing $N^{15}$, that the nitrogen in these compounds was incorporated into both haemoglobin and plasma proteins. This labelled nitrogen was present mainly in the unessential amino acids contained in these proteins. It had also been shown much earlier by Foster, Schoenheimer and Rittenberg (1960) that isotopically labelled nitrogen is readily incorporated into amino acids synthesized *in vivo*.

Rechcigl, Loosli and Williams (1957) showed that various nitrogen-containing materials were utilized by young growing rats to meet the non-essential amino acid requirements. An examination of their results suggests that diammonium citrate was somewhat better than urea for this purpose, but the differences were not very striking. Rose, Smith, Womack and Shane (1949) and Lardy and Feldott (1950) also showed that the non-essential amino acid part of the diet for young rats could be derived from relatively non-specific types of nitrogen compounds.

### *Utilization of Non-specific Nitrogen for Synthesis of Non-essential Amino Acids*

The literature dating back three or four decades contains reports dealing with the use of non-protein nitrogen in chick diets. The general

conclusion reached in most of the earlier papers was that the bird could not make effective use of this type of nitrogen. In the light of later scientific developments it is fairly evident that the diets used were deficient or limiting in some of the essential amino acids, and the results obtained are, not, therefore, surprising. More recently, Featherston, Bird and Harper (1962) published conclusive evidence showing that the young growing chick can effectively utilize non-protein nitrogen in substances such as urea or diammonium citrate to meet the non-essential amino acid requirement. Addition of nitrogen in either form to chick diets containing only essential amino acids at levels insufficient to meet the total nitrogen needs of the chick significantly improved growth, feed efficiency, and nitrogen retention, and gave increased plasma levels of amino acids. Diammonium citrate appeared to be superior to urea in promoting growth. Even though this type of nitrogen compound significantly improved the results, it was not quite as effective in improving feed efficiency as a mixture of non-essential amino acids. Nevertheless, it has been clearly demonstrated that the chick is similar to other single-stomach animals in its ability to utilize non-specific nitrogen for synthesis of the non-essential amino acids.

Scott and associates at the University of Illinois have also shown that the nitrogen required by young chicks in excess of that contained in the essential amino acids, can be met by using glutamic acid or diammonium citrate. Urea was not as effective in their studies for this purpose as the ammonium salt (unpublished results).

At the recent Poultry Science Association meeting, held at Athens, Georgia, in 1965 Young, Griffith, Desai, and Scott (1965) reported results obtained in experiments with laying hens, where a part of the dietary nitrogen was supplied either by glutamic acid or diammonium citrate. In their studies it was found that reducing the protein in the diet for laying hens from 16 to 13% caused a decrease in egg production. In the low protein diet containing both soya bean meal and fish meal, where diammonium citrate and glutamic acid were added to supply the equivalent of 3% protein, this was as effective in restoring egg production level as the control treatment. When the low protein diet did not contain fish meal, increasing the nitrogen in the diet to a level equivalent to 16% by means of diammonium citrate gave better egg production than was obtained when glutamic acid was used to supply a similar amount of nitrogen. These results are difficult to understand, in view of the similarity in effectiveness of these two compounds when they were used in studies with growing chicks and young rats. It is important to recognize that the diets used by Young *et al.* (1965) were practical type rations composed of cereal grains and protein supplements as ordinarily used in feed manufacture. Apparently the lower level of dietary protein contained in the ingredients used, included adequate quantities of the essential amino acids required for egg production.

In studies conducted at Washington State University, during the past three years, glutamic acid fermentation by-products containing large proportions of non-protein and non-amino acid nitrogen have been used. We have found that approximately one quarter of the dietary protein in laying rations could be effectively replaced by such compounds. In these studies, the protein supplied by cereal grain and soya bean meal was replaced so as to maintain the diets isonitrogenous. In a number of the experiments, slightly higher egg production was obtained by replacing a part of the dietary protein with these fermentation by-products, which contain significant quantities of ammonium nitrogen, amino acid nitrogen, as well as cellular protein. This observation would suggest that the diet for layers should contain some non-specific nitrogen. The improvements are small, yet they have occurred in a number of our own experiments, as well as in other laboratories. We are presently engaged in studies to determine whether factors other than the essential amino acid content of the diets and the chemical structure of the non-protein nitrogen are relevant to effective utilization of non-specific nitrogen by poultry.

In some studies, where single substances such as diammonium citrate or diammonium phosphate have been used to supply non-protein nitrogen, the results were poorer than those obtained when the fermentation by-products containing non-specific nitrogen were used. In experiments with growing turkeys, we found that a mixture of glutamic acid fermentation by-product and concentrated Steffen's filtrate, replacing soya bean meal, gave equivalent growth of turkeys between 8 and 20 weeks of age. In contrast, in other experiments, where either diammonium citrate or diammonium phosphate was used, the results obtained were not equivalent to those of the control diet. Actually, the addition of diammonium phosphate to the diet for growing turkeys caused a very striking depression in growth rate. In one experiment, the diammonium citrate appeared to be utilized, but the growth rate fell short of that of turkeys fed the control ration.

### *Effect of Other Dietary Components on Nitrogen or Protein Utilization*

We should not disregard the very real possibility that utilization by poultry of amino acids, as well as the utilization of non-specific nitrogen, is influenced by other components in the diet. For example, Chalupa and Fisher (1963) have shown that replacing a single carbohydrate source with a mixture of sugar, starch and dextrin in a purified type of diet improved protein utilization. It has also been shown by Womack, Marshall and Parks (1953) that the addition of diammonium citrate to a diet containing sucrose and low levels of essential amino acid nitrogen did not improve the nitrogen balance in young rats. Increasing the amount of essential amino acids greatly improved the nitrogen

balance, indicating that they were deficient in the diet. When corn dextrin was used in place of sucrose, some surprising results were obtained. Nitrogen retention by rats fed the low level of essential amino acids was greatly improved to the point where it was equivalent to that of rats fed the sucrose diet with the increased level of essential amino acids. In one series, increasing the nitrogen intake, using diammonium citrate in the presence of sucrose, did not produce nitrogen equilibrium. In contrast, when corn dextrin was used, similar intakes of total nitrogen gave slightly positive nitrogen balance.

Other investigators have reported an influence on chick growth of the carbohydrate in the diet. Dietrich, Monson and Elvehjem (1952) showed that chicks fed a diet containing corn dextrin grew more rapidly than similar chicks fed a diet with sucrose instead. Their results also showed some factor extractable from the dextrin by 70% ethanol that would promote growth when added to the sucrose-containing ration. In contrast to the results of Dietrich, *et al.* (1952), we have recently found in our laboratory, in studies involving differently treated soya bean meal, that diets containing either glucose or sucrose supported more rapid growth than similar diets containing corn starch. Whether this effect is related to specific amino acids or the type of protein used or to unknown factors is not clear at this time. Preliminary experiments showed that methionine supplementation above 0·5% or changing the dietary protein level from 22·5 to 17·5% did not eliminate the differences between starch and sugar containing diets.

If we are to make maximum use of the information showing that poultry are able to utilize non-protein or non-specific nitrogen, the following developments or extensions to our knowledge must be accomplished:

1. *More complete information on the amino acid and total nitrogen requirement of different types of poultry such as young chicks, growing chicks and laying hens.*

2. *More detailed information on composition of feed ingredients.* In addition to the variation in data on amino acid or other nutrient content of feed ingredients, there are many voids in our information on composition. This is particularly true for some of the less common feed ingredients and for many of the nutrients that have not appeared to be of practical significance or importance in the past. In addition to this, it is very probable that we shall need to have more specific information on the content of specific carbohydrates present in feeds.

3. *Additional information on nutrient availability.* We have only limited data on availability of specific amino acids and other nutrients present in commonly used feed ingredients. If we should increase our dependence on non-specific nitrogen to meet the protein needs of poultry, it will be much more important to know how much of a particular essential amino acid in a given feed ingredient is nutritionally available. This will be related to the necessity to make more exact calculations of

essential amino acid content of diets to which we add non-specific nitrogen.

If we proceed to increase our dependence on non-specific nitrogen in poultry production, we shall undoubtedly encounter many other factors which influence the utilization of such nitrogen. For example, it has already been shown by Finlayson and Baumann (1956) that growth of rats fed diets containing urea, diammonium citrate, ammonium carbonate or single amino acids was greatly influenced by the feeding pattern. When the daily food containing the supplemental nitrogen had to be consumed in a limited time (spaced fed) instead of on an *ad lib* basis, growth was greatly depressed and the level of blood urea nitrogen was greatly elevated.

Another circumstance which may be of great importance when such materials as urea are being used in the diet, is the presence of the enzyme urease. Inadequately heated soya bean meal containing active urease may release significant amounts of ammonia from the urea and cause ammonia toxicity.

In the foregoing account I have purposely avoided dealing with such nutrient components as might have been suggested by the title, since such factors as energy or calories, environmental temperature, body size, etc., are discussed by other speakers in this Symposium. Moreover, I am convinced that we have ahead of us a potentially great opportunity for utilizing simple forms of combined nitrogen in meeting the protein needs of poultry, if we embark on new programmes having this as their primary objective.

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# 12

# FACTORS AFFECTING PROTEIN REQUIREMENTS OF LAYERS

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*Synopsis*

Protein requirements can best be compared in terms of the response curve relating egg output to protein intake. A classification is suggested, which recognises four types of effect on responses to protein, depending on whether maximum egg output and/or efficiency of protein utilization is affected under different conditions.

From the data available management and environmental factors are apparently without effect on protein utilization and requirement is in proportion to output. Variation in requirements of different strains is apparently related to output if body weight differences are taken into account. Evidence is presented which indicates a decline in efficiency of protein utilization with increased energy intake and increasing age in the first laying year. Under these conditions requirements will not be in proportion to output.

Economic factors can be taken into account by equating the marginal value of egg output at different levels of protein intake with the marginal cost of protein at different levels of dietary inclusion.

## *Introduction*

PROTEIN requirement can be defined either as the percentage of a complete diet that consists of protein or as the amount of protein consumed daily. In this paper 'protein requirement' refers to the former definition and 'protein intake requirement' to the latter. The two are of course related to food intake. Hence:

$$\text{Protein requirement} = \frac{\text{Protein intake requirement} \times 100}{\text{Food intake}}$$

The word requirement implies that there is a level of protein beyond which further increases have no effect on performance. However, in common with many biological situations protein input-output relationships in the laying hen are generally curvilinear and it is often difficult to arrive at the most widely applicable requirement. What is more important is that a 'requirement' which leads to maximum output may not be the correct one to use. An optimum requirement will have to be defined in terms of a ratio between some measure of input and

output. Quisenberry (1965) suggests that the ratio between feed protein and egg protein, the 'protein conversion' concept, is suitable. Conversion in this sense is maximized at the protein level at which a line drawn through the origin of the protein input/output curve intersects the curve at a tangent. More appropriate is an economic ratio which is optimised at the protein level at which the marginal cost of the protein is equal to the marginal value of the output.

### *Food Intake*

Protein requirement—that is the percentage requirement—is clearly affected by factors which change either protein intake requirements or food intake. Some of the most obvious and important factors operate through the latter mechanism only. Energy level of the diet, environmental temperature and bird size are frequently quoted examples.

The present purpose is not to review the control of food or energy intake in the laying hen. It is sufficient to note that if protein intake requirement is known and differences in food intake can be predicted then the formulation problem is solved.

### *Relationship between Protein and Rate of Output*

Most of the factors which may affect the response to protein will also have direct effects on production itself. Thus the general problem of variations in protein requirement can be studied by looking at the relationship between rate of output and the level of protein intake required to support it under different conditions.

So far as protein nutrition of the laying bird is concerned output can be considered as the sum of three processes of expenditure, egg production, growth and maintenance. This is an oversimplification of the real situation especially insofar as growth and maintenance of body tissue of different composition is concerned. However, in the absence of adequate quantitative data about the metabolic fate of protein consumed by the laying bird such a simple scheme will have to suffice. For comparative purposes in this paper output is considered to be comprised of only egg material, but some account must be taken of body weight and growth differences.

We can thus study the effects of different factors on the protein requirements of laying birds by comparing the curves relating protein intake to egg output under different conditions. In Fig. 1 four pairs of hypothetical curves of this type are shown which are characterised by the level of output and the efficiency of protein utilization under two conditions, A and B.

In responses of Type I, neither output nor protein utilization are affected. Identical response curves are thus obtained. Type II is similar to Type I except that the maximum output attained with

conditions B is lower than with conditions A. In this case sudden attainment of a flat plateau in response is perhaps more likely than a continuing curvilinear response.

In Type III responses the lower output resulting from conditions B persist at all levels of protein input. The final possibility in this classification is Type IV where the conditions lead to the same maximum output level but use protein with different efficiencies. No response of this type has been reported although a comparison of a low and high quality protein fed at different levels might be expected to produce such a set of results.

Such a classificatory scheme is neither exclusive or exhaustive. It does, however, appear to describe usefully what little data is available on this problem and also allows generalizations of the two important conclusions, namely the effect of different factors on requirement itself and the nature of the correlation between rate of output and requirement. Responses of Types I and III lead to the same estimate of

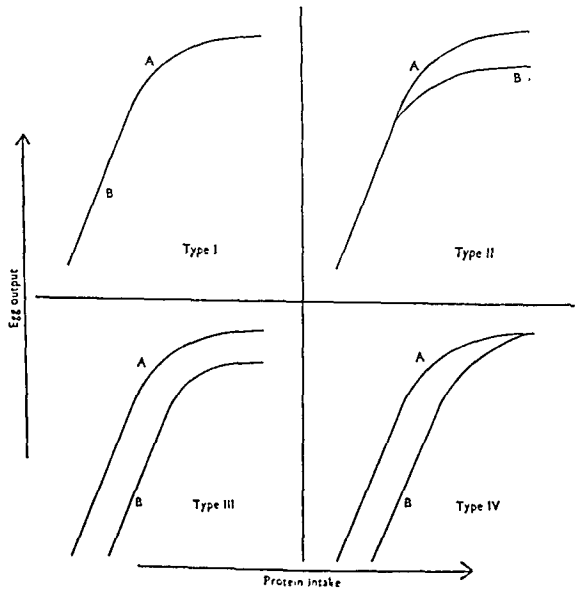


FIG. 1. Hypothetical input-output relationships for protein intake and egg production.

requirement whilst Types II and IV lead to different estimates. If responses of Types III or IV are common the widely assumed correlation between level of output and requirements would not be found under these conditions. The type of data required to apply this classification to different situations is, in many respects, totally inadequate but that which is available is reviewed below.

The discussion is largely based on response curves derived from published data. The curves are fitted to treatment means by normal least-squares procedures. Since each curve is generally defined by only a few points it is not possible to choose between different types of curves by using tests of significance. Instead a choice between a straight line or parabola was made by observation of the points. No other possibilities were considered. Similarly the comparisons made between curves can only be based on observation, statistical comparisons being meaningless with so few degrees of freedom.

### *The Effects of Management on Protein Requirement*

Of all the many variable factors under this heading only two can be documented. The protein requirements of birds housed in cages and in floor pens have been compared by MacIntyre and Aitken (1959), and Thornton and Whittett (1960). Only the data from the second experiment of MacIntyre and Aitken can be used to determine which type of response is involved. These authors fed isocaloric diets containing 10·9, 13·1 and 15·8% crude protein and recalculation of their results leads to the responses shown in Fig. 2. At all dietary protein levels the birds in cages produced less egg material, but when expressed on a protein intake basis, the response curves are seen to be essentially coincident. Since linear responses were found over the range of intakes, the overall pattern is not established. Nevertheless, it seems reasonable to describe the response as either Type I or Type II depending on whether the maximum outputs to be obtained with these systems differ at non-limiting levels of protein intake.

The work of Bray, Jennings and Morris (1965) indicates the effects of light patterns on protein responses. Wide differences in age at sexual maturity were induced by using 'step-up' and 'step-down' lighting patterns in the rearing period. The early maturing birds showed relatively poor egg production and reduced body weight and egg size typical of precocious pullets (Morris & Fox, 1960). Protein requirement was determined by conducting a ten week assay involving dietary protein levels ranging from 7 to 14·5% crude protein. The response curves obtained during the last two weeks of this assay are shown in Fig. 2*b*. The output of the early maturing birds reaches a clear maximum of 44·8 g per bird day and the regression line for this flock refers only to output below this level. The response in this case is clearly of Type II.

Differences in production due to management factors are generally difficult to anticipate. The limited data available suggests that where they are of sufficient importance to justify different rations then protein requirements will probably be correlated with output.

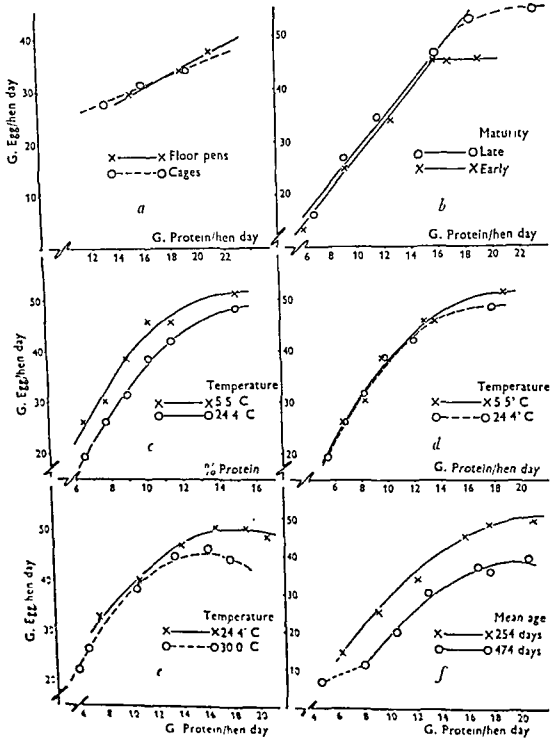


FIG. 2. Responses in egg production, grams per day, to dietary protein level or protein intake under different conditions.

*a*. Different management conditions. MacIntyre and Aitken (1959).

*b*. Different light patterns in rearing stage. Bray, Jennings and Morris (1965).

*c*, *d*, *e*. Different environmental temperatures. Bray and Gesell (1961).

*f*. Different ages. Jennings *et al.* (1964).

*The Effect of Environmental Temperature on Protein Requirements*

The effects of environmental temperature on the protein requirements of laying birds have been examined by Heywang (1947), Heywang, Bird and Vavich (1955) and Bray and Gesell (1961). The data from the last of these clearly illustrates that the effect is completely explained in terms of food intake.

In this work White Leghorn pullets were fed corn-soya diets diluted with starch to give a range of protein levels, for a period of 8 weeks. In a first experiment responses at 5·5°C and 24·4°C were compared. A higher protein percentage was required at the higher temperature (Fig. 2*c*) but the response to a given level of protein intake was not affected by the temperature difference (Fig. 2*d*). When the temperature was raised to 30°C the maximum level of output reached was depressed when compared with 24·4°C. However, this difference in output disappeared at limiting levels of protein intake indicating a response of Type II (Fig. 2*e*).

This is the only evidence available of a factor affecting response to protein with and without affecting maximum output. Since responses of Types I and II are essentially the same, this single example suggests that the type of response obtained is a function of the factor itself and not of the resultant effect it has on output.

Bray and Morrissey (1962) have analysed seasonal patterns of performance at adequate and marginal levels of protein and found that the major trends were apparently associated with the effects of environmental temperature on food intake.

The question whether temperature variations in the U.K. are great enough to suggest the use of 'seasonal' rations appears not to have been investigated. If a single diet is found to be adequate for production over a whole year, then it must provide sufficient intakes of all nutrients at times when food intake is at its lowest and at other times excessive. Thus, the value of using 'seasonal' rations is the saving in cost it entails if the required nutrients are combined with the number of calories that will be consumed at each season.

To investigate the magnitude of the saving for one set of nutrient intakes and feed costs, least-cost diets, which provided these nutrients with a range of energy intakes from 200 to 400 kcals (ME per bird day), were formulated. From the resultant rations the daily cost of feeding a bird a diet based on the requisite energy intake as well as the underestimated intakes was calculated. The effects of environmental temperature on caloric intake were estimated from the data of Ota and McNally (1961) which indicate a fall in energy intake of approximately 25 kcal ME per 5°C rise in temperature.

The results suggest that temperature variations likely to occur under intensive conditions in the U.K. are not sufficient to justify the use of 'seasonal' rations. The use of two rations is likely to reduce feeding costs by no more than ninepence per bird per year.

*Genetic Effects on Protein Requirements*

Most of the reports of genetic variation in protein or amino acid requirements refer to the growing chick. Breed or strain differences in growth response to amino acids have been reported by Hegsted, Briggs, Elvehjem and Hart (1941) for arginine and glycine, by McDonald (1957), Miller, O'Barr and Denton (1960), Hess, Edwards and Dembnicki (1962) and Lepore (1965*a*) for methionine, by Nesheim and Hutt (1962) and Griminger and Fisher (1962) for arginine, and by Enos and Moreng (1965) for lysine.

In many of these cases amino acid levels were expressed as a proportion of the diet and no data on food consumption were provided. It is possible that the genetic differences are due to variations in food intake and not in amino acid requirements expressed on an intake basis. This seems to be the explanation of the differences in arginine requirement, since Nesheim, Christensen, Arnold and Hutt (1964) have reported that the difference in growth on arginine-deficient diets between lines selected for high and low arginine requirement disappeared when arginine intake was equalised in pair-feeding or force-feeding experiments. More specifically the difference may be one of response in food consumption to amino acid deficiency or imbalance, as the growth difference also disappears when an arginine-deficient crystalline amino acid diet was used instead of a casein diet. Lepore (1965*b*) reaches the same conclusion about genetic differences in response to a methionine deficient diet. The data of Slinger, Sibbald and Pepper (1964) show a real difference in protein utilization between a fast and slow growing strain of birds when fed a single protein level, but this is not evidence of a difference in requirement.

If large genetic differences in protein utilization are found to be common then there is no basis for predicting requirements from a consideration of output characteristics. In this case responses must be determined separately for each strain and unless the range of strains available becomes very small, exploitation of such differences in requirement is unlikely to be of practical importance. The exception would be in the rather unlikely circumstances that it was in breeders' interest to select for different protein requirements.

Of greater potential interest is the possibility that differences in protein requirement are quantitatively associated with differences in output characteristics and can, therefore, be predicted. At the present time it is impossible to anticipate whether any differences in protein requirements that may exist between commercial laying stocks are due to specific genetic differences or are simply associated with the output characteristics.

Comparisons of the protein requirement of different strains in a single experiment have been reported by Harms and Waldroup (1962), Sharpe and Morris (1965), Moreng, Enos, Whittett and Miller (1964)

and Thornton and Whittett (1960). In the last of these, egg weight is not reported.

Harms and Waldroup (1962) report a significant strain × protein level interaction for egg production when two similar strains of White Leghorn pullets were fed 13, 15 or 17% crude protein. In Fig. 3*a* the relationship between egg output and protein intake for these two strains is shown. The similarity of these curves suggests that the response to different protein intakes was identical for these strains and the interaction arose because of different responses in food intake. Examination of the points in Fig. 3*a* shows that protein consumption is identical for

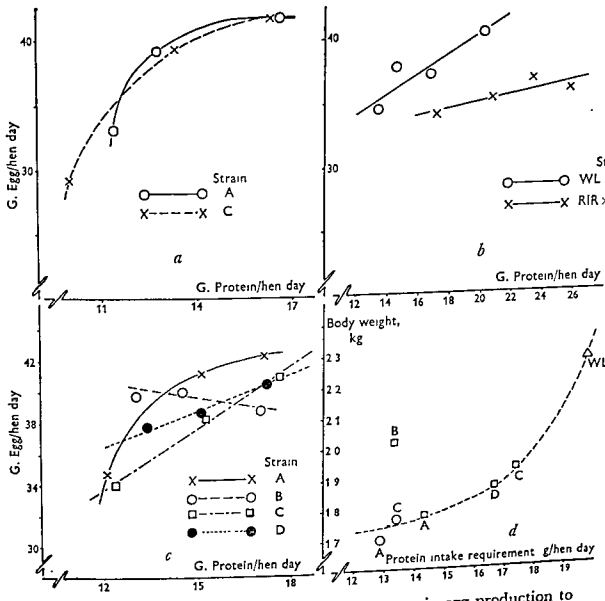


FIG. 3. Effect of strain genotype on responses in egg production to protein intake.

Response curves of different strains, after:

*a*. Harms and Waldroup (1962).
*b*. Sharpe and Morris (1965).
*c*. Moreng *et al.* (1964).
*d*. The relationship between calculated daily protein intake requirement of different strains for 40 g egg production per bird day and body weight. Data from Harms and Waldroup (1962) ○, Sharpe and Morris (1965) △, Moreng *et al.* (1964) □.

the two strains at 17% protein but is higher in Strain A at lower levels of protein. This leads to a lower percentage protein requirement in Strain A. It is interesting to note the similarity of these results to those of Nesheim *et al.* (1964) on arginine requirement and of Lepore (1965*b*) on methionine requirements. In all cases genetic differences in requirement arise because of differences in the response in food intake to conditions of protein deprivation.

Sharpe and Morris (1965) compared responses in a Rhode Island Red × Light Sussex strain and a small White Leghorn-type hybrid. These strains differed in egg output and also very substantially in body weight. The response data shown in Fig. 3*b* indicate that the response curves are quite separate but the exact nature of the difference cannot be determined. For an output of 35 g egg material per day the larger bird appears to need some 4·5 g extra protein. Part of this at least will be required to maintain the 1·1 kg of extra body weight and to cover extra growth requirements (3·95 g and 1·78 g liveweight gain per day). Whether this is a quantitatively sufficient explanation of the difference cannot be decided.

Moreng *et al.* (1964) fed three levels of protein to groups of twenty-seven birds from four unspecified commercial strains. The response curves calculated from their data are shown in Fig. 3*c*. The most obvious difference amongst the strains is that Strain B shows no response to protein over the range 13-17 g protein per day whilst the other three strains show clear positive responses. The level of output reached by Strain B is similar to the other strains and it appears that this strain makes more efficient use of dietary protein for egg production. This conclusion is emphasized by the fact that this was the largest of the four strains.

To try and compare the data from these three experiments the protein intake required to support 40 g egg output per day has been calculated from the fitted response curves in Fig. 3. The data from the Rhode Island Red × Light Sussex strain used by Sharpe and Morris (1965) was excluded as this strain failed to reach this level of output and extrapolation is unjustified. The estimates varied from 12·9 g to 19·6 g protein per bird day and, of course, many reasons could explain these differences. However, with the exception of Strain B of Moreng *et al.* (1964), the protein intake requirement for 40 g egg per day was found to be closely related to body weight (see Fig. 3*d*). Final body weight data, rather than the average, had to be used as these were the only figures available in some cases. When similar calculations were made for 36 g egg material both strains used by Sharpe and Morris could be included but two strains from Moreng *et al.* (1964) were excluded. A good relationship was again obtained, the curve having a similar slope. This higher protein cost for larger birds is to be expected since maintenance and, to a lesser extent, growth requirements are increasing. However, a straight line fitted to the data in Fig. 3*d* has a

slope of about 10 g protein per kg liveweight. The published estimates of protein requirements for maintenance are very variable but are all substantially less than this.

The summary of Brody (1945) suggests 0·5 to 0·7 g digestible protein per kg per day as a basal metabolic requirement. From feeding trial data Halnan (1939) proposed 1·3 g digestible protein per kg per day and Leveille and Fisher (1960), 1·75 g digestible protein per kg per day. The latter data were obtained with roosters. More recently Squance and Brown (1965) have summarized the available data on endogenous nitrogen losses. Their own data yields a figure of 0·83 g digestible protein per kg per day. In a summary of various feeding and metabolism experiments Morimoto, Kubota and Ariyoshi (1961), propose a protein requirement for maintenance of 1·1 g protein per kg per day.

Even allowing for the variability in these data, for protein quality and for digestibility as low as 50% it appears that the relationship between protein requirement for egg production and body weight shown in Fig. 3*d* is not entirely explained by increased requirements for maintenance. Differences in daily growth rate in the data used were all less than 1 g and for all strains maximum output lay between 40 and 42 g per day, so it seems unlikely that other protein expenditure can account for this. Finally it is possible that the relationship is fortuitous. This possibility should act as a spur to the collection of more and better data on this important question.

There does, however, seem to be some hope that general relationships between output characteristics of different strains and their protein requirements do exist. Encouragement for this conclusion is found in an extensive study by Griminger and Scott (1959) of the relationship between growth rate and lysine requirements. These authors obtained differences in growth rate of genetic origin both by selecting fast and slow growing fractions from a single population and by using two strains with different growth rates. In both cases percentage lysine requirement was found to be unaffected by growth rate but recalculation of their results on a protein intake basis shows that the differences in growth rate disappear at limiting levels of lysine intake, giving responses of Type II. Under these conditions lysine intake requirement is related to growth rate.

Several authors have assumed a simple linear relationship between output and intake requirements and have used this to predict requirements of birds of different kinds. The most important study of this type is that of Combs (1960, 1962), who partitioned the variation in methionine intake amongst birds on a methionine limiting diet into separate parts associated with average body weight, body weight change and egg output. The partial regression coefficients obtained were rounded off to give estimates of the methionine intake required to support one unit of activity in the various processes.

His final equation was:

$$M = 0{\cdot}05W \pm 6{\cdot}2\Delta W + 1{\cdot}135E$$

where $M$=methionine requirement (mg per hen day)
$W$=average body weight in grams
$\Delta W$=average body weight change (g per hen day)
$E$=average egg output (g per hen day)

This requirement can be related to a feed intake predicted by the equation given by Byerly (1941) or to predicted energy intake as derived by Combs (1960, 1962).

Byerly's (1941) equation is:

$$F = 0{\cdot}523W^{0{\cdot}653} \pm 1{\cdot}126\Delta W + 1{\cdot}135E$$

where $F$=food intake (g per bird day)
and $W$, $\Delta W$ and $E$ are defined as above.

If these equations are used to predict percentage methionine requirements for birds of different body weights, the calculated differences turn out to be surprisingly small.

In Table 1 the calculated percentage methionine requirements of birds weighing 1·4 kg and 2·7 kg for different levels of output are given.

TABLE 1

*Predicted methionine requirements as related to body weight and egg production (after Combs, 1960)*

| Body weight kg | Daily egg production (g per hen-day) | | | | |
|---|---|---|---|---|---|
| | 30 | 35 | 40 | 45 | 50 |
| | Per cent methionine | | | | |
| 1·4 | 0·236 | 0·248 | 0·259 | 2·68 | 0·277 |
| 2·7 | 0·228 | 0·237 | 0·245 | 0·253 | 0·260 |

Growth is assumed to be zero. The body weights represent the likely extremes of variation in commercial laying birds and output will normally vary from about 35 to 45 g egg per bird day. Over this range the difference in requirements of the two types of birds differ by only 0·01%. The differences due to egg production are only about 0·02% so it appears that if protein and amino acid requirements, and food intake are both correlated with output characteristics and these equations are even approximately correct, then variations in percentage requirements of different strains may turn out to be only small.

*The Effect of Age and Stage of Lay on Protein Requirement*

As birds age egg output declines, and this has led to a widely held belief that protein requirements decline as the laying year progresses. This belief presupposes that increases in maintenance requirements will

not cancel out the decrease in production requirements, a problem that is easily answered in theory, and also that the utilization of ingested protein for productive purposes takes place with equal efficiency at all ages—a more difficult problem.

Experiments involving a change in protein level as the year progresses have been reported by Coligado and Quisenberry (1961), Quisenberry (1965), Sharpe, Morris and Fox (1965) and Owings (1964), but before reviewing these the proposed decrease in requirement should be investigated.

In various experiments at the University of Reading it has been observed that the differences in output between adequate and inadequate protein levels tend to increase or to remain the same as the laying year progresses. This observation is confounded by the effect that feeding a deficient diet for increasing lengths of time will have on body reserves and so an attempt was made to determine independently the requirement for protein at the beginning and end of the laying year.

A report of this work was presented by Jennings, Fisher and Morris (1964). Protein response assays were conducted over the periods 30-40 weeks and 60-70 weeks of age and a comparison made of data for the last two weeks in each assay, referring to mean ages of 254 and 474 days. The response curves reproduced in Fig. 2*f* are clearly of Type III.

A possible explanation of this displacement of the curve is the extra maintenance requirements of older birds, but in fact the average difference in between the old and young birds was almost zero at this stage of the assays. An upper limit to the effective difference is given by the body weights at the start of the assays but even these differed by only 200 g. The older birds were losing body weight more rapidly during the assay but if this loss contributes to the protein needs of egg production it reinforces the conclusion that the utilization of dietary protein for egg production is much less efficient in birds at the end of the laying year as compared with young pullets. In fact it appears that very similar levels of intake are required to support maximum output in spite of the differences in level that this entails.

The conclusion seems to be that although output declines and maintenance requirements apparently differ only slightly, the requirement for protein remains the same for birds at different ages. There are two possible explanations for this. Either the efficiency of utilization of ingested protein declines in some or all of the many processes involved or alternatively protein requirement is related not to the level of output but to the overall need to keep the metabolic functions operating at a maximum.

An attempt to confirm these observations at several ages and for methionine- and lysine-limited proteins is currently being made (Fisher, unpublished evidence). To date a comparison of the responses to methionine levels from 0·187% to 0·312% and to lysine levels from

0·4% to 0·65% at 21-31 weeks and 31-41 weeks of age has been completed and a preliminary analysis of the results carried out. An untidy response in egg numbers during the first period makes interpretation of the output data difficult but the patterns of response in egg weight show considerably increased sensitivity to both methionine and lysine level in the second assay. In Fig. 4 the changes in egg weights on three of the lysine levels are shown. From 21-31 weeks egg weight increases almost normally at all levels of lysine, but when the diets were introduced to birds at 31 weeks following a period of adequate protein nutrition large and persistent differences in egg weight soon appear. It cannot be argued that differences in egg production are responsible, since the birds producing eggs in the early period will have high individual rates of lay even though average production is low. Body weights were similar at both periods. It appears that a change in lysine requirement for maximum egg weight can be demonstrated for two age groups as similar as these. The data on methionine requirement lead to the same conclusion.

Milton and Ingram (1957) also report greater percentage protein requirements for hens than for pullets but no details of this work are available.

It is thus not surprising that it has not been found possible to reduce the level of protein as the laying year progresses unless excesses of protein have been fed initially.

Data presented by Coligado and Quisenberry (1961) and Quisenberry (1965) show inconsistent patterns of response both to different levels of protein fed constantly and to reduced protein levels and do not clearly demonstrate reduced protein requirements with age. Owings (1964) showed that protein level could be reduced as the year progresses

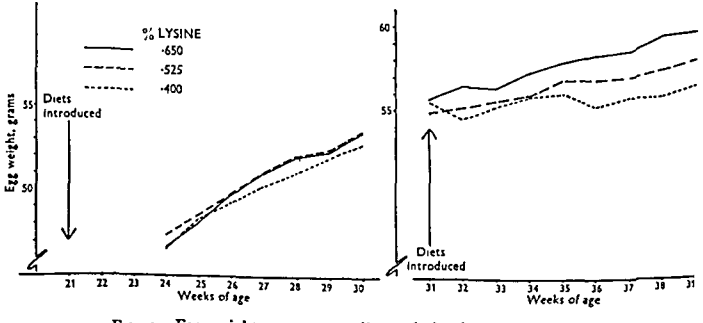


FIG. 4. Egg weight responses to dietary lysine level from 21 to 31 weeks and from 31 to 41 weeks of age.

but failed to show that the lower levels of protein were inadequate if fed constantly. Sharpe, Morris and Fox (1965) made all possible changes between 14 and 10·5% crude protein diets after 12 and 24 weeks of lay. With a White Leghorn hybrid bird the effect of feeding the low protein diet constantly or of changing on to it at either age was always to depress production by a similar amount.

It is concluded that protein requirements do not decrease as the laying year progresses in a way that a consideration of output would suggest and thus reductions in protein level are not justified on present evidence.

Some of the data from experiments at the University of Reading show response to dietary changes *per se*. By elevating protein levels after 12 weeks of lay Sharpe, Morris and Fox (1965) obtained greater egg production than that supported by feeding either diet alone. Unpublished data show the converse effect, that changing protein level down leads to lower egg production than feeding the low level constantly. Jennings and Morris (1965) have noted a striking pattern of 'compensatory' production following a period of protein deprivation of varying degrees.

### *The Effect of Dietary Energy Level on Protein Requirements*

The adjustment of protein and amino acid levels for variations in energy content of the diet according to a suitable energy to protein ratio (see Combs, 1962, for review) is now common practice and requires no further description.

This principle rests on two assumptions which have not been widely investigated. First it is assumed that the adjustment of food intake on diets of different energy content is precise and secondly that the protein intake requirement is not affected by energy level.

Two experiments conducted at the University of Reading by Sharpe (1961) indicate that the second assumption may not be correct. In the

TABLE 2

*Output obtained from two strains fed 10·5% crude protein at two energy levels (Sharpe and Morris, 1965)*

| | Energy content of diet kcal ME per kg | |
|---|---|---|
| | 2165 | 2760 |
| RIR×LS | | |
| Protein intake (g per day) | 17·9 | 17·1 |
| Energy intake (kcal per day) | 368·0 | 441·0 |
| Egg output (g per day) | 37·04 | 34·07 |
| WL Hybrid | | |
| Protein intake | 13·4 | 13·9 |
| Energy intake | 282·0 | 345·0 |
| Egg output | 38·68 | 34·58 |

experiment reported by Sharpe and Morris (1965) a range of protein levels were fed in diets containing about 2760 kcal ME per kg. The lowest protein level (10·5%) was also fed in a diet with 2165 kcal ME per kg. Some results for two strains of bird fed this low level of protein at two energy levels are shown in Table 2. In both strains protein consumption is similar on both diets but egg output considerably higher at the lower energy level. A similar result was obtained when a range of protein levels were fed at three energy levels (Sharpe, 1961) and is also to be found in the results of Lillie and Denton (1965).

This effect of energy on protein metabolism is presumably due to the extra calorie intake rather than the energy level of the diet but beyond this observation no explanation can be offered.

*Economic Factors affecting Choice of Protein Level*

It was noted above that the problem of choosing a protein level will usually be solved optimally in economic terms. This involves bringing into a statement of requirement a consideration of output value. For a single measure of output and a single input variable the optimum rate of input is defined by conventional marginal analysis as the level at which the marginal cost of the input and the marginal value of the output are equal. This means that generally either differences in value of the output or in costs of the input will cause variations in the optimal level.

In the present context the marginal value of eggs produced at a given level of protein intake is the slope of the output value-protein intake curves. Thus information about the shape of the response curve and an equation for it are prerequisites for this type of analysis. This problem is discussed in general terms by Monroe (1954) and Dent (1964). Once a suitable response function is obtained its slope at any point and thus the equation for marginal value is given by the first derivative of the function.

The determination of the marginal cost of part of a ration is not generally possible. However, if the ration at each point is defined as 'least-cost' for a given set of conditions by making use of a linear programme then the cost of unit increase in one part of the ration can easily be determined. The calculation must of course be made for each set of conditions.

The theoretical basis at least is thus available for determining an optimal economic level of protein. Where linear programming techniques are used for formulation there is no difficulty in computing rations with a given marginal cost structure. The determination of suitable production functions presents some problems but it should be possible to collect sufficiently useful data of this sort. Dent (1964) discusses the use of this principle for more than one dietary factor and presents a preliminary application of the technique to pig nutrition.

As an illustration this technique has been applied to determine the optimum protein intakes of a flock of birds at two ages using the data in Fig. 4 for the derivation of production functions. Egg output was assumed to have a value of 4s. per kg and a parabolic curve was fitted. This yielded for the young birds

$$y = -0{\cdot}990 + 0{\cdot}309x - 0{\cdot}0072x^2$$

and for the old birds—

$$y = -0{\cdot}656 + 0{\cdot}2043x - 0{\cdot}0038x^2$$

where $y$=value of eggs produced (pence per bird day)
$x$=protein intake (g per bird day)

Thus the marginal value expressions are $(0{\cdot}3096 - 0{\cdot}144x)$ and $(0{\cdot}2043 - 0{\cdot}0076x)$ for the old and young birds respectively.

A linear programme was set up to formulate the daily ration for one bird. Each ration provided exactly 400 kcal ME which was the average consumption of the experimental birds at high protein levels. Protein was defined exactly in grams/day and protein quality maintained by using minimum levels of amino acids as a percentage of the protein. The marginal values of eggs produced for young and old birds at different levels of protein intake and the marginal cost of the protein at 1-g intervals are presented in Table 3. The data show that intakes

TABLE 3

*Marginal cost of protein and marginal value of output from young and old birds (see text)*

| Protein intake g./day | Marginal cost pence/day | Marginal values Young birds pence/day | Old birds pence/day |
|---|---|---|---|
| 14 | 0·0029 | 0·1080 | 0·1094 |
| 15 | 0·0056 | 0·0936 | 0·0907 |
| 16 | 0·0056 | 0·0792 | 0·0721 |
| 17 | 0·0175 | 0·0648 | 0·0534 |
| 18 | 0·0175 | 0·0504 | 0·0384 |
| 19 | 0·0175 | 0·0360 | 0·0162 |
| 20 | 0·0175 | 0·0216 | −ve |
| 21 | 0·0175 | 0·0072 | −ve |

of about 19 g per day and 20-21 g per day are optimal for the old and young birds respectively. These essentially similar optima are to be expected since the response curves are approximately parallel.

Clearly this simple analysis has not established feeding levels of any general value. The general applicability of the experimental data, the suitability of the simple parabola used, the relationship between the experimental rations and the formulated rations with respect to protein quality and the economic data used would all have to be considered carefully before such a result were obtained.

However, the example demonstrates a logical procedure for choosing

a protein level for any given set of conditions which simultaneously takes into account variations in ingredient supply and price, output value and in responses. It could find early application in defining optimum levels of use for synthetic amino acids and perhaps more generally once the effort involved is judged to be worth while. A consideration of the cost of nutritional precision is very pertinent to a discussion of factors which affect nutrient requirements.

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# DISCUSSION ON PART IV

*Dr W. Bolton (Edinburgh):* Both speakers have dealt with interactions and three types have been mentioned: interactions of the bird with the food, of the food with the purse, and of the protein with the other dietary components.

(a) *Interactions of the bird with the food.* Mr Fisher dealt with the effects of egg production and body weight on the protein requirement, and one thing seems clear: either we can supply the geneticists with a given diet and tell them to breed birds to perform optimally on it; or we can tell them to produce the birds and we'll find out what diet they need for optimal production.

For light-bodied birds, Hill (1956) has shown that the food intake is largely controlled by the metabolizable energy content of the food. With heavier-bodied birds this control tends to be less well-developed; this is seen particularly with broiler breeding stock—the heavier-bodied layers are intermediate. Mr Fisher's paper showed that this principle cannot be applied to protein requirement, so we have a problem to solve and more research is indicated.

(b) *Interactions of protein with other dietary components.* The requirements for lysine (Schwartz, Taylor & Fisher, 1958) and for methionine (Baldini & Rosenberg, 1955) have been shown to be increased when the dietary level of metabolizable energy is increased. The Agricultural Research Council (1963) state that their recommended levels of amino acids apply to a diet containing a stated metabolizable energy content and they recommend that all amino acid contents should vary with the metabolizable energy content. Mr Fisher's paper has shown us that there is a limited amount of evidence indicating that this may not be strictly true, but not sufficient to let us discard the principle at this stage.

(c) *Interactions of the food with the purse.* Diets should be compounded to leave a satisfactory margin of profit in the purse of the compounder and the maximal profit in the purse of the farmer. This was stressed by Mr Fisher when dealing with economic aspects. Prof. McGinnis told us of the profitability of using ammonium salts to meet part of the demand for nitrogen. He showed us that as much as one quarter of the dietary nitrogen in layers' diets has been effectively supplied by non-protein, non-amino acid nitrogen; and that a layers' diet containing 13% protein, supplemented with diammonium citrate to give a crude protein content of 16%, supported good rates of egg production. I find the scientific aspects here are exciting and the economic possibilities fascinating to contemplate.

*Professor G. F. Combs (Maryland):* I should like to present one slide concerning some recent work on the lysine requirement of laying hens.

The lysine needs have been partitioned on the basis of body weight, weight gain and grams of egg produced. This work was done by Mr Owen Thomas, a graduate student from South Africa, with caged layers individually fed and recorded. We have eliminated from the data those which did not lay during seven consecutive days throughout a two-week collection period. The following formula was derived from data of two such collection periods in the fourth month after egg production began in the experimental diets.

*Equation* 1. $L=0{\cdot}0377W\pm 8{\cdot}61\Delta W+12{\cdot}56E+467(R-r^1)-63{\cdot}9$

Then when $r^1=0{\cdot}36$ the equation is simplified and gives

*Equation* 2. $L=0{\cdot}038W\pm 8{\cdot}61\Delta W+10{\cdot}63E+467R-168{\cdot}1$

$L$=available lysine requirement/hen/day in mg.
$W$=average body weight for period in grams.
$\Delta W$=average daily change in body weight in grams.
$E$=average grams of egg product/hen/day.
$R$=ratio of available lysine to energy in diet expressed as mg of lysine per metabolizable kilocalorie.
$r^1=0{\cdot}36$ (ratio of lysine to energy in diet containing 0·534% available lysine, expressed as mg of available lysine per metabolizable kilocalorie)

And then by further simplification and addition of the modified Byerly equation one gets an available lysine requirement prediction equation in terms of % of diet.

*Equation* 3. $$\%\ \text{lysine}=\frac{C(0{\cdot}04W+8{\cdot}6\Delta W+10{\cdot}6E)}{4536T(1{\cdot}45W^{0{\cdot}653}\pm 3{\cdot}13\Delta W+3{\cdot}15E)}$$

Where $C$=kilocalories of met. energy per lb.
$W$=average body weight in grams.
$\Delta W$=average daily weight change in grams.
$E$=average grams of egg produced/hen/day.
$T$=appropriate temp. correction factor (e.g. $T=0{\cdot}98$ for autumn, 1·05 for winter, 1·0 for spring and 0·94 for summer)

*Mr C. Fisher (Reading):* I don't feel very happy about the use of this type of equation for prediction under different conditions. Two assumptions in particular don't withstand critical examination. First, we know that the relationship between nutrient intake and egg output is not a linear one yet in these equations we only find a linear term with respect to egg production. If we consider a strain of birds of given size and, for example, we want to calculate from this equation how much more lysine we might give to increase egg output by 2 grams, then if we are on the diminishing part of the response curve to lysine, as is likely at this time, we will seriously underestimate the amount that would be required to effect this increase in egg output. The second objection is that we don't yet know whether a single set of coefficients

is applicable under all conditions. For example, it is clearly not justifiable to use a single set of coefficients to compute the lysine or protein requirements of birds of different ages, since our data suggests that the responses are quite different. I think we ought to look more closely at the theoretical basis of these equations before wider use is made of them.

*Professor Combs:* I am aware of the problem of using linear equations for functions that are not really linear. We feel that the curvilinear relationship of lysine intake and egg production is due primarily to the changes in utilization of lysine with level of intake. For this reason, we inserted a component ($R-r^1$) in the original equation to correct this difference. $R$ is defined as milligrams of lysine per kilocalorie and $r^1$ is the $R$ term for the level which gave optimal egg production. Thus $R-r^1$ gives a zero component at optimal production levels and permits one to eliminate it in a simplified equation. This also permits a linear relationship instead of one which is not linear.

*Dr T. C. Carter (Edinburgh):* I take issue with Mr Fisher over the first of his remarks. I think it is important that attempts should be made to produce equations such as the one we have seen, because it is a valuable summary statement of the experimental data. It should be recognized as such and should therefore be discarded as soon as an equation that gives a better fit with the data has been produced. This will probably entail introducing further parameters. I have one question: Can Professor Combs explain why some of the parameters in his equation appeared both in the numerator and in the denominator?

*Professor Combs:* This is probably due to my limited mathematical abilities. However, it is because of a combination of two separate equations; had I had time to develop this I would have shown an equation for lysine needs in terms of milligrams per bird per day which, as I attempted to point out, is simply the upper portion in brackets only. The lower portion in brackets is the modified Byerly equation for feed requirement, converted to give an answer in metabolizable kilocalories. So really all I wish to emphasize seriously is the upper portion of the equation, which gives the lysine need based upon maintenance, weight change and egg production. We are not at all pleased with the weight change term because most hens, after they reach their peak of nitrogen retention, will continue to gain weight on a good diet, but just maintain, or even lose, nitrogen. One has a complicated situation in attempting to relate either apparent positive or negative gains to changes in lysine needs.

*Mr W. R. Muir (Glaxo Laboratories):* I support the view that requirement does decline with age.

*Mr Fisher:* I believe the data reported by Shapiro and Fisher (1965) do not support the conclusion that protein intake requirements decline with age. In this work birds of unspecified age were fed a constant daily allowance of 16 g egg albumen protein for four months. The data show that there was a decline in mean nitrogen retention from

853 mg per bird/day in the first month to 738 mg per bird/day in the fourth month. The authors then use other data obtained with (presumably) younger pullets to infer that a N retention of 738 mg per bird/day could have been obtained in the fourth month by feeding 13 g egg albumen protein per bird/day. There is no direct demonstration that either egg production or N balance would have been maintained for these older birds if only 13 g protein had been fed. This paper does not, I think, contribute to the present question of whether the coefficients relating protein input to egg output are the same in birds of different ages.

*Dr G. D. Rosen (London):* May I ask Professor McGinnis whether he has measured the effect of a citrate salt or citric acid inclusion, bearing in mind the comparative results reported for diammonium citrate and phosphate?

*Professor J. McGinnis:* We haven't tried to assess any change of energy requirement in relation to this but if we take away the energy that normally would be supplied with the nitrogen in a protein material, then we must replace it in the diet.

*Mr W. R. Muir (Glaxo Laboratories Ltd.):* Professor McGinnis has given us a lot to think about. I fear however that we already find a certain amount of difficulty in reaching the required levels of essential amino acids in poultry rations and when we do reach these levels, we usually have, I think, a surplus of non-essential amino acid nitrogen in the ration.

I have some difficulty in finding a biochemical interpretation of some of Mr Fisher's statements. I think the efficiency of protein utilization in his experiments was about 40%, whereas Professor Brown, yesterday, was talking about utilisations of 50-60%. Obviously, when discussing protein requirement, the biological efficiency of the feed protein has to be taken into account and this is one fact that must be borne in mind when considering Mr Fisher's experiments.

It is commonly said that the falling production of the pullet as it passes through its first year of lay is due to advancing age, but I find it difficult to believe that such a bird is ageing in a biochemical sense. It is certainly still a young bird in comparison with the normal life span of the fowl.

It is possible that the apparent increase in protein requirement for maintenance as the bird increases in age may—apart from the effect of increasing weight—be due to the accumulation of body fat which I suspect may increase the birds need for 'protein'. Nevertheless, I much prefer to be guided in future by the work done by Shapiro and Hans Fisher (*loc. cit.*) which does show that, as egg production falls, the protein requirement does drop from about 16-17 grams of a particular protein mixture down to about 13 grams.

This and other observations encourage me to think that we should give serious consideration to phase feeding of the laying pullet.

May I revert to the interesting point of the correspondence of the amino acid pattern of the mixed proteins of wheat and of the pattern of amino acid requirements for egg production. It occurs to me that even when additional nitrogen as ammonium citrate is added to wheat the level of 'protein' and the protein-energy ratio is likely to be too low for the laying bird especially as the food mixture is also diluted with minerals. On the other hand, wheat might be reinforced with wheat protein mechanically concentrated from wheat flour and I regret that I have not had an opportunity to work and collaborate in this field. It seems to me that this reinforced wheat protein diet—supplemented perhaps with a little lysine—which we hope to get at 5s. per lb one day—might make just the right sort of diet for the laying hen.

### *Closing Remarks*

*Dr H. Temperton (Harper Adams College, Newport, Shropshire):* Professor Parkes, Chairman of the Scientific Advisory Committee, unfortunately could not be with us for the end of the conference. In addition to acting as your chairman for this afternoon's session, it is my pleasant duty to wind up these proceedings, and I will do so as briefly as possible commensurate with my responsibilities.

The organization of this Symposium, I think you will probably agree, has been timely. We are very satisfied with the attendance. As far as we can ascertain, the maximum number present at any session has been 140, which is considerably more than on the last occasion, and I think would have been even greater except for the restriction on the number that could be accommodated residentially. In selecting this particular theme, the Scientific Advisory Committee was in no doubt about the breadth and depth of its scientific interest. I was very pleased that, at a rather late stage in the proceedings, Mr Fisher and Dr Bolton also reminded us of the tremendous economic importance of the subject that we have been discussing in its various aspects over the last two days. The organizing committee did not anticipate that these deliberations would result in any firm conclusions. The objective of the exercise was to define rather more clearly to us the outstanding problems in this field, and if it has done no more than that, then I think the Symposium will have been well worth while.

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*Entries in italics refer to papers in this volume. References made by authors to their own work are not separately indexed.*

# SUBJECT INDEX

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